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Reprinted from The American Journal of Anatomy
Vol. 34, No. 3, January, 1925

AN EXPERIMENTAL AND MORPHOLOGICAL STUDY OF THE PARATHYROID GLANDS OF THE CAT

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THREE PLATES

INTRODUCTION

The problem of the functional significance of the parathyroid glands has for many years attracted the attention of anatomists and physiologists. Many interesting theories of parathyroid function have been promulgated and many important facts accumulated, but the fundamental problem of the rôle of these glands in the economy of the organism is almost as much of a mystery to-day as it was thirty years ago.

In the present study, attention has been concentrated upon the cat. This animal was chosen not because it is the most favorable material for parathyroid work, for in fact it is greatly inferior to the dog, but because of the many conflicting statements in the literature regarding the effects of parathyroid removal in this animal. By using a large number of animals and correlating the anatomical and physiological data, it was considered likely that the discrepancies in the earlier work could be satisfactorily explained, especially in view of the fact that some of the conclusions drawn from the older work were based upon a very limited number of cases.

An historical review of the literature would be profitless here, as this has been admirably covered by several authors.

[1] The experimental work was done at the Osborn Zoölogical Laboratory, Yale University.

Welsh ('98) gives an excellent critical account of the work on parathyroids up to 1897 and Paton and Findlay ('17) have discussed the more recent literature. A very thorough and lucid account of the parathyroid problem is that of Sutherland Simpson in volume 1 of Endocrinology and Metabolism, and a complete bibliography of parathyroid investigations conducted upon mammals is to be found in volume 5 of the same series.

A list of investigators who have utilized cats in their work and a statement of the chief results obtained by each will be found on page 487 of this article. The work of Vincent and Jolly and several more recent workers needs more detailed treatment, however, because of the relation of their investigations to our own.

In 1905 Vincent and Jolly published the results of an extensive investigation upon the physiology of the mammalian thyroid and parathyroid. They used a large number of animals of various species in the experiments, viz., cats, dogs, foxes, monkeys, rats, guinea-pigs, and rabbits. We are concerned here only with the work dealing with cats. In these animals they found that when either one or both thyroid lobes and one parathyroid was left in situ, the cats showed no signs of tetany. When the parathyroids alone were left, out of five cases one died. In this they concur with other investigators. When, however, the thyroid was left intact and all four parathyroids successfully destroyed, five out of fifteen animals recovered. One surviving animal did not show typical symptoms. In two fatal cases the thyroid at postmortem was found to be extensively damaged. Vincent and Jolly concluded that in a great majority of cases no ill effects of any kind resulted from the removal of the parathyroids per se. According to them, simple parathyroidectomy in the cat, when the thyroid lobes are left uninjured, is not necessarily fatal, the cases with fatal termination being those in which they had done most damage to the thyroid. In 1906 they published a second paper confirming their former findings.

These conclusions are decidedly at variance with the prevailing view. The opponents of Vincent and Jolly endeavored to explain the comparatively large number of cases that showed no symptoms after removal or destruction of all four parathyroids as probably due to the presence of accessory tissue, which had been left behind at the operation. Indeed, Harvier and Morel ('09) state that 50 per cent of all cats examined possessed accessory glands; however, in the paper in which this statement occurs no data are given as to the number of cats actually examined, as the paper is concerned with the data obtained from less than five animals. We have been unable to consult Harvier's ('09) paper.

Paton and Findlay ('17) used a large number of cats in their experiments—forty-seven in all—and described in some detail the symptoms following parathyroid extirpation. Their work, although an extremely important contribution to the problem of the parathyroids, adds little to the question of accessory glands in the cat because the majority of the cats operated upon by these investigators were either killed or else decerebrated, before marked tetany symptoms supervened, for utilization in experiments involving measurements of the electrical excitability of the peripheral nerves. It is impossible to determine from their data just what per cent of the animals would have succumbed following gland removal, for it is probable that many of the animals used for measurement of the electrical excitability of the peripheral nerves exhibited only transient tetany and probably would have recovered had they been permitted to live.

Farner and Klinger ('20) have also performed extensive experiments upon the cat. They regard accessory parathyroid tissue as being practically universally present in this animal, as 20 per cent of their cases failed to show any tetany symptoms. Others probably recovered, but no data are given on this point.

Recently, Shapiro and Jaffee ('23) have reported their findings in the examination of the thoracic thymus of forty-two cats. Their method, which they admit to be crude, was

to section small pieces of the thoracic thymus. While the very crudity of the method employed emphasizes the frequency of accessory glands, their conclusions must, at least for the present, be limited in application to the particular form studied. Their evidence is not sufficient to permit the generalization stated in their conclusion, viz.: "Accessory parathyroids are present in man and animals in a large percentage of cases. By careful examinations of serial sections of the neck and thorax they probably could be demonstrated in almost every case" ('23, p. 723). A complete supplementary study of the accessory parathyroids in the dog (Marine, '14, found 4 to 6 per cent) will be necessary before their criticisms of Dragstedt, Luckhardt, and their co-workers will be justified. The dangers of too wide a generalization in the present stage of our knowledge of parathyroid function are self-evident.

It has been shown (Nicholas and Swingle, '24) that in cats accessory parathyroid tissue was present in 35 per cent of the cases. The further studies reported here show the same percentage and have further emphasized for us the large element of chance which has been such a confusing factor in parathyroid experimentation.

Our experiments show that an animal may survive when accessory parathyroid tissue is present and that complete parathyroid extirpation in the cat under normal conditions of life and diet generally (probably invariably, though our data are not absolutely conclusive on this point) results in death. The possibility of death resulting even though accessory tissue is present, but insufficient to compensate functionally for the loss of the normal glands, cannot be entirely disregarded because several such cases have occurred in our work.

A fatal insufficiency is produced by a complete parathyroidectomy in approximately 60 per cent of cats. When the operative field is extended so as to include most of the probable locations of accessory parathyroid tissue, the number of fatalities increases.

The physiological importance of accessory parathyroids is shown indirectly by the increase in the percentage of death occurring when these bodies are removed and also directly by the removal of accessory glands in an animal which showed no tetany symptoms for twelve days after the removal of four parathyroids. This animal died three days after the second operation in violent tetany.

The above factors lead us to believe that in the three cases in which recovery occurred (seventy-seven cases examined) accessory tissue was probably present, even though this was not found at autopsy.

ANATOMY

A. Methods

The principal anatomical data were secured by examining fresh thyroid and parathyroid material under the high-power binocular microscope, as it was received from the operator at the time of extirpation. As such observations were necessarily confined to tissue removed from the field of operation, they were supplemented by study of injected and sectioned material.

Two injection masses were employed: one, ordinary chrome yellow was injected through the femoral artery immediately after death; this brings out clearly the main arterial circulation of the thyroid and parathyroids. Figure 1 was drawn from such a preparation. The second series of animals was injected with an ammoniacal carmine solution. The cats were etherized and the solution injected into the inferior cava; the abdominal aorta was severed and clamped so as to permit a slight flow of blood from the proximal end; this is helpful in determining when the injection is complete. When the heart action finally ceased, pressure was applied by means of a syringe. The thyroparathyroid apparatus was then removed and placed in a fixative consisting of 10 per cent formalin plus 5 per cent acetic acid. The acid precipitates the carmine and the precipitate is insoluble in subsequent

reagents. The material was dehydrated and cleared in Spalteholtz's fluid (50 per cent benzol benzoate and 50 per cent methyl salicylate).

In material cleared in this way, all the larger blood vessels are clearly shown and, in addition, the finer vascularization is well brought out. The thyroid tissue appears pink in contrast to the deep red of the vessels ramifying in it, whereas the parathyroids appear white against the pink background. The transparency of the cleared thyroid lobes enables the internal parathyroids to be located easily.

B. Gross structure of thyroid and parathyroid apparatus

The thyroid lobes of the cat are located on either side of the trachea just below the level of the cricoid cartilage and extend downward over the first five or six tracheal cartilaginous rings. In general, there is no isthmus connecting the two thyroid lobes in adult cats. In some instances, however, a thin band of thyroid tissue has been found connecting the two lobes at their inferior poles; presumably this represents remains of the isthmus which is said to be present in very young kittens and during foetal life. Figure 6 d is a diagram of the thyroparathyroid apparatus of a half-grown cat, showing the remains of the isthmus.

The anatomy of the thyroparathyroid apparatus of the cat was first worked out by Kohn ('95). Since his paper, other investigators have figured the thyroids and parathyroids showing their normal relationships in various animal species. Vincent ('22) shows in some detail the commoner variations which are to be found in the anatomical relation of the parathyroids to the thyroid lobes in the cat.

In Kohn's classic description of the glands of the cat and other animals, four parathyroids are said to be the typical number; two of these are referred to as external parathyroids (Epithelialkörper IV), and two as internal parathyroids (Epithelialkörper III). Kohn's terminology is derived from the embryological origin of these glands, the third visceral cleft giving rise to the internals, the fourth cleft to the externals.

The external parathyroids of the cat are generally located at the superior pole of the thyroid lobe on the anterior surface. Their location, however, is by no means constant (fig. 6), as we have found them in practically any position upon the anterior surface of the thyroid. The external parathyroid is usually considerably larger than the internal. Externals varying from 3 mm. to 7 mm. in length have been observed. The number of external parathyroids in the cat is also subject to variation. There may be three or more externals upon one thyroid lobe and but one external upon the other (fig. 6). As many as four or five supernumerary glands may be found on one thyroid lobe, but this condition is rare, occurring in but two cases out of a total of seventy-seven animals.

The internal parathyroids of the cat are smaller and vary in location and number more than the externals. They may be found anywhere upon the tracheal surface of the thyroid lobe or even enclosed within its substance. Some of the variations in position of the internal parathyroids met with in our work are indicated in figure 6.

The arterial supply of the parathyroids of the cat is from the thyroid artery which enters the thyroid lobe at its superior (cephalic) pole. This artery evidently corresponds to the superior thyroid artery of other species, as the inferior thyroid artery is lacking in the cat. Immediately after entering the thyroid it gives off several small branches, one or more of which supply the parathyroids. We have noted several variations in the venous supply to the glands. In most cats a single vein drains both thyroids and runs down the ventral surface of the trachea emptying into the right or left jugular.

The nerves to the parathyroids apparently consist entirely of sympathetic fibers from the perivascular plexuses. However, we have made no special effort to ascertain the origin and distribution of the nerve elements of the thyroid and parathyroids, as this has been fully covered in the excellent papers of Andersson ('94), Rhinehardt ('12), and others.

Kohn ('95) was the first to call attention to the intimate relation existing between the thymus and thyroids of the cat. In these animals, a portion of the cervical thymus is, in many cases, found attached to, or even partially embedded in, the thyroid lobes. In adult cats the cervical thymus tissue may be lacking or replaced by fat, but in the younger animals some thymus is generally present in close connection with the thyroids and external parathyroids.

The presence of these small masses of thymus tissue so far anterior in the cervical region inclines us to the belief that complete thymectomy in the cat is an exceedingly difficult operation and practically necessitates extirpation of the thyroid lobes also, in order to be complete. Removal of the thoracic thymus of the cat is certainly not to be regarded as a complete thymectomy.

Years ago, Harvier and Morel ('09) called attention to the necessity of removing the cervical thymus in the cat for complete parathyroid extirpation, because they found accessory parathyroid tissue in this structure. Our experience has offered abundant confirmation of this fact, and while we cannot agree that the cervical thymus contains accessory tissue in 50 per cent of cats, the occurrence of accessory glands is so frequent that the removal of cervical and upper thoracic thymus and cervical fat is certainly justified in any operation dealing with parathyroid extirpation in the cat.

The derivation of the parathyroids from the epithelium of the third and fourth branchial clefts and their intimate association in development with the anlagen of the thymus, together with the shifting and migration of tissue which occurs in the cervical region of mammalian embryos, greatly increase the possibility of forming accessory parathyroid glands, and of their inclusion within some portion of the thymus not only in cats, but in other animals as well.

EXPERIMENTAL

Methods

The technique involved in removing the parathyroids of the cat is quite simple, and with a little practice it is possible to remove thyroids, parathyroids, cervical fat, and thymus with practically no bleeding except a slight ooze from the subcutaneous vessels. It is less difficult to extirpate the thyro-parathyroid apparatus of kittens than of adults, owing to the thinness of the overlying muscle layers and fascia in the younger animals.

The incision was median, extending from the thyroid cartilage to the sternum. This is an extensive cut, but is necessary if the cervical thymus and fat are to be entirely removed, and it is recommended that these structures be extirpated along with the thyro-parathyroid apparatus because of the large per cent of cats possessing accessory parathyroids which are generally located in or near them. In the earlier operations of our series the precaution of removing all of the cervical thymus and fat was not taken, and as a result a large number of animals failed to develop tetany despite the removal of the usual four parathyroids. Later, following the suggestion of Harvier and Morel ('09) and our own anatomical investigations, the field of operation was extended to include the cervical thymus and fat, and the number of animals developing fatal tetany increased.

After the location of the thyroid lobes, these were dissected free from the trachea, the blood vessels ligated with silk thread, and the glands removed. The wound was closed by suturing separately, 1) the deep muscle layer, 2) the subcutaneous tissue, and, 3) the skin. The usual aseptic precautions were employed and ether was used for anesthesia; no bandages were applied to the wound. Following the operation, the cats were placed in clean metal cages, the floors of which were covered with fresh sawdust. Of a total of sixty-two operations, but two animals died of infection, although five others developed slight infections from which

they recovered within a few days or died of tetany before the suppuration spread. Three of the seven infected animals were cats which had been reoperated upon. In several cases it was found necessary to reoperate four or five times upon a single individual within a few weeks in order to remove accessory parathyroid tissue left behind at the previous operations. Reoperated individuals were very susceptible to infection.

We attribute the small number of infections to the irrigation method employed in postoperative treatment; following operation, the wound was irrigated thoroughly several times a day with Dakin's fluid, by inserting the nozzle of the irrigation apparatus between the lower stitches. This was done regardless of whether the animal did or did not show any indications of infection.

It is much simpler to remove thyroids and parathyroids together than to attempt their separation and removal of one or the other alone. We have attempted several such operations, but in the cat it is an exceedingly difficult task to remove the parathyroids leaving the thyroid intact. The external parathyroids are easily removed or separated from the thyroid, but the internals, because of their minute size in many animals and variable position on the tracheal surface of the thyroid lobes, are difficult to identify and extirpate with certainty. The operation of removing the thyroid lobe leaving the parathyroids with intact blood supply we regard as impossible in the majority of cats, regardless of the condi tions in other forms. A glance at figure 1 is sufficient to convince one of the difficulty of removing the thyroid lobes without practically destroying the blood supply of the external and internal parathyroids. In rare instances one and sometimes both of the external glands are so situated that it is possible to leave them with undisturbed blood supply upon removal of the thyroid lobes. One such case is described later in this paper. The difficulty lies in the fact that the arterial supply to the parathyroids consists of minute vessels from the thyroid artery, which arise from that vessel and

are found in or upon the substance of the thyroid gland (fig. 2).

Cautery has not been employed in our work because the parathyroid glands are destroyed before they can be positively identified by microscopic examination and the thyroid is always injured by this method.

The recurrent laryngeal nerve of the cat is so situated in relation to the thyroid lobes that it need give no concern to the operator if the thyroid is carefully dissected free from the tracheal fascia with the blunt edge of the scalpel before extirpation. We have had two fatalities, attributable in part at least to injuries of the recurrent laryngeal nerve. The abbreviated protocols are given below.

Protocol cat 18

August 4. Thyroparathyroidectomy. Female, weight, 1240 grams. All parathyroids identified. Cat recovered from anesthesia within one hour. Breathes with great difficulty—stridor. Refuses food. Runs about in a lively fashion.

August 5. Cat found dead in cage at 8:30 A.M. Autopsy showed inclusion of the right recurrent laryngeal nerve within the ligature of the thyroid vessels.

The second case was similar, except that the animal lived for two days following the operation and did not refuse food. The animal breathed with great difficulty and vomited several times, finally dying in convulsions. Autopsy showed the left recurrent laryngeal nerve to be severed. Whether or not death in these two cases was due solely to injuries to the recurrent laryngeal, it is impossible to say, but the symptoms and autopsy findings support such a view.

Thyro-parathyroidectomized cats are very susceptible to conjunctivitis, and this is especially true of kittens. An effective method of treatment which was employed consisted of bathing the eyes several times a day with 1–2000 solution of silver nitrate or a weak solution of argyrol.

Complete thyro-parathyroidectomy. Removal of all parathyroid tissue in the cat, usually, if not invariably, results

in death. It is impossible to assert without qualification that cats cannot live without parathyroids, because several animals have been observed which survived what apparently was a complete parathyroidectomy, careful search at necropsy failing to reveal any accessory glands. It is our opinion, however, that accessory parathyroid tissue was probably present in these animals despite the negative postmortem findings. This is discussed more fully in the section devoted to accessory glands. Two symptom complexes, tetany and depression, result from gland removal, both of which are typical reactions of the organism to parathyroid insufficiency.

Just what constitutes typical tetany in cats is not easy to state precisely, as the symptomatology is protean in character and transient atypical symptoms peculiar to the individual constantly arise. The major symptoms of tetany in the cat are well known and have been so completely described by earlier workers (see especially Vincent and Jolly, '05, '06, and Paton and Findlay, '17) that a detained account of tetany is unnecessary here. Two shortened protocols given below are typical of cats dying of tetany.

Protocol cat 15

August 3. Thyroparathyroidectomy. Female, weight, 820 grams. All four parathyroids identified.

August 4. Refused food; tremors of head and body muscles; shakes paws when walking as if they were covered with water; extensor spasms of hind legs; rapid respiration; violent tremors; marked twitching of back and flank muscles; animal prostrate and mews continually; unable to stand erect; several epileptiform convulsions within half an hour, in which the cat lost consciousness and became rigid; died in convulsion, weight 770 grams. Autopsy showed no parathyroid tissue.

Protocol cat 26

August 15. Thyroparathyroidectomy. Female, weight, 1470 grams. All four parathyroids identified.

August 16. Eats well; shakes paws when walking; tremors of body muscles; stiff in hind legs.

August 17. Animal improved; eats liver; slight tremors.

August 18. Tetany symptoms reappeared; paw shaking, marked tremors; extensor spasms noted; animal greatly depressed.

August 22. Repeated epileptiform convulsions ending in loss of consciousness and rigidity; marked cerebellar symptoms; animal unable to coordinate its muscular movements and staggers about drunkenly or may run backward.

August 23, 24. All symptoms worse. Constant rapid tremors and twitchings involving most of the body and head muscles. Epileptiform attacks with opisthotonos; animal moribund and killed on this date; weight 1170 grams. Autopsy showed no trace of parathyroid tissue.

Probably the most constant and characteristic symptom of parathyroid insufficiency in cats is paw-shaking. Practically every investigator working with these animals has commented on this peculiar reaction in which the cat appears to be trying to shake water from its paws. This together with stiffness of the hind legs is one of the earliest symptoms to appear in operated cats.

We have noted in a large majority of our cats deprived of the parathyroids a characteristic symptom which, so far as we are aware, has never been described. The conjunctiva of the nasal angle of the eye (nictitating membrane) is drawn upward and across the cornea in such a manner that the eyes seem to diverge as in external strabismus. The layer of conjunctiva forms a film over the inner corner of the eye which in marked cases may extend half-way across the pupillary area and seriously interfere with vision. The symptom may be masked in some animals, owing to pigmentation of the conjunctiva. Figure 3 represents the condition described. Etherized cats show a similar condition, but the conjunctiva (nictitating membrane) promptly retracts to its usual position when the anesthetic is withdrawn. In parathyroidectomized cats, however, the condition is permanent and persists even after death. In animals suffering from transient symptoms of parathyroid insufficiency, but which later return to normal, owing to the possession of accessory glands or to unknown causes, the 'eye sign' is present so long as the untoward symptoms persist, but disappears when the animal

TABLE 1[1]

ANIMAL NO.	AGE	SEX	DURATION OF EXP. IN DAYS	WEIGHT BEGINNING	WEIGHT END	OPERATION LOSS OF WEIGHT	DIED (D), KILLED (K)	CAUSE OF DEATH: T, tetany; D, depres'n	OPERATION	GLANDS IDENTIFIED				SYMPTOMS FOUND										REMARKS
										Exter.		Inter.		Accessory	Tremors	Eye sign	Paw shaking	Epileptiform at'ks	Cachexia	Cerebellar symp.	Spasticity	Laryngismus	Depression	
										R	L	R	L											
1	⅔	♀	4	1550			K	T	C	+	+	+?	—?	—	+	—?	+	—	+	+	+	—	+	
2	A	♀	12	2100	2150	+50	K		C	+	+	+	+	+	—	—	—	—	—	—	—	—	—	
3	⅔	♀	14	1270	1080	150	D	*		+	+	+	+		+	—	sl	—	—	—	—	—	—	Grafted autoplastic (4) parathyroids. No tetany. Pneu.–death.
4	K	♀	4	440	390	50	D	T	C	+	+	+	+?	+	+	+	+	+	—	++	+	—	+	
5	½	♂	28	1260	1540	280	K		*	+	+	+	+	—	+	—	+	—	—	—	+	—	+	Grafted autoplastic (2) exter. parathyroids. Died–removal transplant.
6	½	♀	2½	1450			D	T	C	+	+	+	+	—	+		+	+	+	+	+	—	+	
7	⅔	♀	30	1500	1040	460	D	T	C*	+	+	+	+	3	+	+	+	+	—	+	+	—	—	Reoperated (2). Final operation complete.
8	½	♀	4	1200	1100	100	D	T	C	+	+	+	+	—	+	+	+	+	+	+	+	—	+	
9	A	♀	36	2270	2355	85	K		*	+	+	+	+	3	+		+	+	+	—	+	—	—	Grafted (4) parathyroids. Pregnant. Sl. tetany–reoperation for removal of graft when 3 accessories were found.
10	A	♀	26	2350	2650	300	K			+	+	+	+	—	±	tr	—	—	—	—	±	—	±	
11	A	♀	4	2180	2030	150	D	D		+	+	+	+	—		sl			+				+	Thyroid engrafted. No effect on tetany.
12	A	♂	29	1720	2000	280	K			+	+	+	+	3	±	++	sl	—	—	—	sl	—	tr	Accessory glands preserved life in 12.
13	⅔	♀	13	1690			D	TD		+	+	+	+	—	+	+	+	+	—	+	+	—	+	
14	A	♂	5	3000	2720	280	D	D	C	+	+	+	+?						+				+	
15	⅛	♀	1	820	770	50	K	T	C	+	+	+	+?	—	+		+	+	—	+	+	—	—	

16	A	♂	19	3890	3970	80	K			+	+	+	+	1+	+	+	+	+	—	+	+		+	Had tetany but not fatal. Animal died under ether during reoperation for accessories.
17	⅔	♀	21	1780	1340	340	D	T	C*	+	+	+	+	2	+		+	—	—	—	+	—	+	Reoperated.
18	½	♀	1	1240			L	*	C	[2]+	[2]+	[2]+	—?									+		Recurrent laryngeal nerve included in ligature.
19	A	♀	2	1750			K	T	C	+	+	+	+	—	+	+	+	+	—	+	+	—	+	
20	½	♀	4	960			K	T	C	+	+	+?	+?	—	+	+	+	+	—	+	+	—	+	
21	½	♂	2	1110	1045	75	K	T	C	+	+	+	+	—	+	+	+	+	—	+	+	—	+	
22	K	♂	11	840	490	350	D	TD	C	+	+	+	+	—	+		+	—	—	+	+	—	+	
23	A	♀	1	2930	2670	260	K	T	C	+	+	+	+	—	+	++	+	+	—	+	+	—	—	
24	A	♀	2	1690	1590	100	K	T	C	+	+	+	+	—	+	++	+	+	—	+	+	—	+	
25	A	♀	9	3080	2600	320	D	T	C	+	+	+?	+?	—	+	+	+	+	+	+	+	—	+	
26	⅔	♀	9	1470	1170	300	K	T	C	+	+	+	+	—	+	++	+	+	+	+	+	—	+	
27	A	♂	8	2000	1590	410	D	T	C	+	+	+?	+	—	+	++	+	+	+	+	+	—	+	
28	yA	♂	3	1660	1440	220	K	T	C	+	+	+	[3]+	—	+	++	+	+	—	+	+	—	—	
29	yA	♀	5	1640	1370	270	D	T	C	+	+	+	+	—	+	++	+	+	+	+	+	—	—	
30	K	♂	3	450	410	40	D	T	C	+	+	+	+	—	—	—	—	+	—	—	+	—	—	
31	yA	♂	9	3090	2270	820	K			+	+	+	+	+?	+	+	+	++	+	+	+	—	+	This animal recovered completely. Ac. gland suspected but not found.
32	A	♂	16	3720	3800	80	K			+	+	+	—?	+	—	—	—	—	—	—	—	—	—	Accessories present. Animal discarded
33	K	♀	13	650	705	55	K			+	+	+	+	sl	—	sl	—	—	—	—	—	—	—	Latent tetany. Accessories present, animal discarded.

[1] Under the heading Age, K indicates a kitten; ½ and ⅔ indicate half grown and two-thirds grown, respectively; yA is a young adult, and A is an adult. A star in either the Cause of Death column or the Operation column refers the reader to special conditions which are treated under the column headed Remarks. When extreme reactions were obtained in certain cases, the effect is noted as 2+. A ± reaction was recorded when slight symptoms were present.

TABLE 1—*Continued*

ANIMAL NO.	AGE	SEX	DURATION OF EXP. IN DAYS	WEIGHT BEGINNING	WEIGHT END	OPERATION LOSS OF WEIGHT	DIED (D), KILLED (K)	CAUSE OF DEATH: T, tetany; D, depres'n	OPERATION	GLANDS IDENTIFIED				SYMPTOMS FOUND										REMARKS
										Exter.		Inter.												
										R	L	R	L	Accessory	Tremors	Eye sign	Paw shaking	Epileptiform at'ks	Cachexia	Cerebellar symp.	Spasticity	Laryngismus	Depression	
34	K	♀	8	550	480	70	D	D	C	+	+	+	+	—	+	+	+	—	—	+	—	—	++	
35	½	♀	7	320	560	260	D	TD	C	+	+	+	+	—	+	+	+	+	—	+	+	—	+	
36	⅓	♂	11	800	853	53			*	+	+	+?	+?	+	—	—	—	—	—	—	—	—	—	Animal discarded. Accessory glands.
37	A	♀	10	2250	1630	620	D	T	C	+	+	+	+	—	+	+	+	+	—	+	+	—	+	
38	⅓	♀	8	600	530	70			*	+	+	+	+	—	+	+	+	+	—	+	+	—	—	
39	A	♀	15	2435	2478	33		*		+	+	+?	+?	+	—	—	—	—	—	—	—	—	—	Accessories present. Animal discarded
40	A	♂	1	1820	1680	140	D	T	C	+	+	+?	+	—	+	+	+	+	—	+	+	—	+	
41	A	♂	12	2600	1000	1600	D	D	C	+	+	+?	+	—	+	++	+	—	+	+	+	—	++	
42	A	♂	7	2475					*	+	+	+	+	+	—	sl	—	—	—	—	—	—	—	Accessories present. Animal discarded
43	K	♂	2	600	565	35	D	D	C	+	+	+	+	—	sl	—	sl	—	—	sl	—	—	++	
44	A	♂	4	1940	1878	62	D	T	C	+	+	+	+	—	+	+	+	+	—	+	—	—	—	Graft experiments–Graft region infected, killing graft–died of general infection.
45	A	♀	3	2400	2370	30	D	T	C	+	+	+	+	—	+	+	+	—	—	+	—	—	+	
46	A	♂	7	2240					*	+	+	+	+	+	—	—	—	—	—	—	—	—	—	
47	A	♂	4	1840	1780	60	D	T	C	+	+	+	+	—	+	+	+	+	—	+	+	—	sl	
48	K	♂	4	450	370	80	D	D	C	[2]+	+	+	+	—	—	—	—	—	—	+	+	—	+	
49	K	♀	3	435	408	27	D	T	C	+	+	+	+	—	+	+	+	+	—	+	+	—	+	
50	K	♀	3	320	290	30	D	D	C	+	+	+	+	—	+	—	+	—	—	+	+	—	++	
51	A	♂	4	2750	2701	49	D	T	C	+	+	+	+	—	+	+	+	—	—	—	+	—	+	
52	K	♂	3	420	398	22	D	TD	C	+	+	+	+	—	+	—	+	—	—	—	+	—	+	

53	½	♂	2	650	600	50	D	D	C	+	+	+	+	—	—	—	—	—	—	+	+	—	++		
54	A	♀	6	1510	1205	305	D	T	C	+	+	+	+	—	+	—	+	—	—	+	—	—	++		
55	A	♀	23	2420	2065	355	K		*	+	+	—?	—?	+	+	+	—	—	—	—	—	—	sl	Guanidine injection. Transplantation parathyroids vitiated by accessories.
56	A	♂	22	5050	4181	869	K		*	+	+	+	+	+	—	—	—	—	—	—	—	—	—	Guanidine injection. Accessories present.
57	A	♀	21	3240	3915	675	K			—?	+	+	+	+	+	—	—	—	—	—	—	—	+	Guanidine injection. Accessories present.
58	A	♂	12	3011	2975	36	K			[2]+	+	[3]+	—?	+	—	—	—	—	—	—	—	—	—	Guanidine injection. Accessories present.
59	A	♂	7	3565			K	T	C*	+	+	—?	—?	—	+	+	+	—	—	+	+	—	+	Homoplastic trans. of (3) paras.	
60	½	♂	7	1210	1150	60	K			+	+	+	+	+	—	—	—	—	—	—	—	—	—	Strychnine injected. Autoplastic transplants of (3) paras. vitiated by accessories.	
61		♂	27							+	+	+	+	+	—	—	—	—	—	—	—	—	—	Guanidine injection. Graft experiment vitiated by accessories.	
62		♂								+	+	+	+	+	—	—	—	—	—	—	—	—	—		

returns to normal. This reaction we have termed the 'tetany eye sign.'

We have rarely observed this peculiar extension of the nictitating membrane in normal unoperated cats, and when it occurs the condition lasts but two or three seconds. However, a well-developed case was observed in an animal following total adrenalectomy; the cat had previously been thyroparathyroidectomized, but had failed to show tetany. The symptom disappeared twelve hours after operation.

Cats vary greatly in regard to their resistance to the lethal effects of parathyroid extirpation, some individuals develop violent tetany and succumb within fifteen or twenty hours following operation; others may remain without symptoms for several days and suddenly within an hour develop a violent and fatal attack of tetany. The survival period of operated cats, in which all trace of parathyroid tissue is removed, varies from twelve hours to twelve days, the usual time being about four and one-half to five days. The reason for such variation is not clear, except that it may be a matter of individual resistance to the toxin or toxins responsible for the tetany syndrome and possibly, in those animals which survive longest, to the presence of minute amounts of accessory tissue.

Complete ablation of the parathyroids in numerous cases fails to induce the typical tetany symptoms. Instead of violent nervous excitation, a peculiar symptom complex may result which has been aptly described by the word depression. The cat upon recovery from ether reacts in a normal manner, eats heartily, and moves about in lively fashion, and to all appearances is as well as before the operation. This condition may last ten or twelve hours or even for several days. The usual tetany symptoms are conspicuous by their absence. However, after a longer or shorter interval of normal activity, individual cats vary greatly in this respect—the animal becomes psychically and physically sluggish, withdraws to one corner of its cage, and refuses to move unless prodded. The cat refuses food and sits for hours at a time

TABLE 2

Parathyroid investigations on the cat[1]

INVESTIGATOR	METHOD	OPERATED	DIED	SURVIVED	REMARKS
Schiff, J. M., '84	Extirpation				Does not give number of cats used. Main work on dogs.
Wagner, J., '84	Extirpation	12	12		Operated 13, but left thyroid (and therefore parathyroid intact) on one.
Smith, J. L., '94	Extirpation	10	6	4	
Vessale and Generali, '95	Extirpation	10	9	1	
Moussu, '96	Extirpation?	17	8	6	Three died accidentally.
Munk, H., '97	Extirpation	14	9	5	
Moussu, '97–'98					
Welsh, '98	Extirpation	5	5		
Jeandelize, P., '03	Extirpation	3	3	0	
Vincent and Jolly, '05–'06	Cautery	18	12	6	
Pineles, '08	Extirpation	17	17		Tried injection on 9 with no effect.
Harvier, '09	Cautery	9	9	0	
Harvier and Morel, '09	Cautery	3	3		
Ott and Scott, '09	Extirpation	60	—	—	No data given.
Trendelenberg, '10	Extirpation	4	4		Performed thyrodectomy, but cats showed parathyroid symptoms.
Carlson, '12	Extirpation	25	—	—	
Morel, '12	Cautery	17	9	5	
Carlson, '13	Extirpation	31	30	1	Ten showed depression (late pregnancy).
Paton and Findlay, '17	Extirpation	44	15	1	27 killed for physiological work. 1 died accidentally.
Farner, E., and Klinger, R., '20	Extirpation	40		20%	No data on deaths. 20% showed no signs of tetany.
Jaffee and Shapiro, '23	Histological only	42			Anatomical studies only on preserved material. (See p. 471.)
Nicholas and Swingle, '24	Extirpation	62	42	20	

[1] These figures as far as possible include only complete operations; i.e., when parathyroids were left intact they are not included in the cases in the table.

without moving and pays no attention to anything going on about it. The 'tetany eye sign' appears, and if the animal is forced to move about, marked cerebellar symptoms become evident, the cat staggers about drunkenly and seems to lack ability to properly coordinate its muscular movements. Death generally supervenes two or three days after onset of the depression. During the interval of depression the cat may show no nervous or muscular symptoms whatever. Generally, however, cats dying of depression also show some tetany symptoms. The typical muscular tremors and twitchings may be present, but are not marked and may entirely disappear some time before death occurs. Mixed symptoms, i.e., animals which present typical tetany alternating with periods of marked depression are numerous, whereas cats showing depression alone following parathyroid removal occur comparatively infrequently in our series.

In the early part of the work, animals dying of depression were regarded as anomalous cases and the symptom complex as an idiosyncrasy on the part of certain individuals to parathyroid insufficiency. The first case was looked upon with suspicion. Other factors beside gland removal were considered responsible in part at least for death. However, as several more cases appeared and as the autopsy findings were consistently negative, it was concluded that death from depression following parathyroid removal is a typical gland-deficiency syndrome in a small per cent of cats. The protocol of an animal dying of depression is given below.

Protocol cat 43

October 22. Thyroparathyroidectomy. Male kitten; weight, 600 grams. All four parathyroids identified.

October 23. Greatly depressed; mews plantively when handled, refuses to move about; refuses food; no tetany symptoms.

October 24. Animal weak; lies prostrate in cage and mews; unable to walk but staggers and falls from side to side; will suddenly topple over while sitting; 'tetany eye-sign' noted; no tremors; unable to sit erect; coma. Died at 8:35 P.M.; weight, 565 grams. Autopsy showed nothing unusual.

Young kittens seem to suffer more from depression following gland removal than older animals. All except two of our fatal cases of depression were young animals, and our records show six such cases, all of the kittens being under ten weeks of age. However, young kittens of this age do develop violent tetany. Probably the most pronounced symptoms of this syndrome in young kittens are the inability to properly coordinate muscular movements (cerebellar symptoms) and weakness. The following protocol is typical for animals presenting the 'mixed' type of reaction to gland removal, i.e., transient tetany followed by fatal depression.

Protocol cat 4

August 16. Thyroparathyroidectomy. Female kitten; weight, 440 grams. All four parathyroids identified.

August 17. Slight tetany; fine tremors of head and back muscles; paw shaking and 'tetany eye-sign' noted; animal ate liver.

August 18. Marked cerebellar symptoms; animal totters about unsteadily; no tremors or twitchings observed; legs weak; depression.

August 19. Fibrillar twitchings of head and body muscles; marked weakness evident; greatly depressed; refuses to move; refuses food.

August 20. Dead; weight, 390 grams; autopsy negative.

Loss of weight is an invariable concomitant of parathyroid insufficiency in cats, and to date we have observed no exceptions to this rule. All of our animals developing tetany lose weight very rapidly, as is well shown by the accompanying table, but it must be borne in mind that cats either in depression or tetany refuse food. Sutherland Simpson remarks (Endocrinology and Metabolism, vol. 1, p. 529) that he has observed that dogs eat meat heartily between convulsive attacks. Cats will sometimes eat small amounts of liver or drink milk after a convulsive attack, but generally they react to food as though it sickened them; they sniff at it and turn away, and may even gag and attempt to vomit. Several animals were forcibly given milk or water by means of a pipette because they were unable to drink.

Removal of the thyroid leaving parathyroids intact. Several investigators have reported successful results following

this type of operation in cats, e.g., Welsh ('98) and Vincent and Jolly ('05–'06), but we have so far been unable to accomplish satisfactorily the removal of the thyroid leaving all parathyroids intact. In the great majority of our animals the technical difficulties involved in such a procedure are insuperable because of the origin of parathyroid blood supply. It is simple enough to remove the thyroid lobes leaving the parathyroids behind, but the blood supply to the glands in such cases is practically destroyed and the operation is really equivalent to an autoplastic graft.

However, one case was found in our series where it proved feasible to remove the thyroid lobe of one side, leaving the external parathyroid with intact blood supply. This was due to the aberrant position of the left external parathyroid which was dissociated from the thyroid lobe and lay anterior to it. The left internal and the right internal and external parathyroids were dissected away from the thyroid and reimplanted into the wound. The animal was kept for twenty-six days and then killed for examination. The transplanted parathyroids could not be located, but the left external gland which had been left in situ with intact blood supply was in excellent condition. The protocol in abbreviated form follows:

Protocol cat 10

July 27. Thyroidectomy with left external parathyroid left intact and right external, left and right internals reimplanted; adult female; weight, 2350 grams.

July 31. Slight tetany; refuses food; marked 'tetany eye-sign'; paw shaking and tremors noted.

August 4. No tetany symptoms; eats heartily.

August 12. Is normal in every way; gaining in weight.

August 22. Killed for examination; weight, 2650 grams. No trace of thyroid tissue or implanted parathyroids could be found. Intact parathyroid in good condition.

This case offers clear evidence that loss of the thyroids in the cat induces no untoward symptoms; instead the animal gained 300 grams in weight and was normal in every respect.

The transient tetany symptoms were due (vide seq. under discussion of transient tetany in operated animals) to slight parathyroid insufficiency following destruction of the blood supply to three of the glands. Welsh ('98) and Vincent and Jolly ('05) demonstrated that if one parathyroid gland is left behind at the operation, the cat remains normal or develops only mild symptoms of tetany.

Thyro-parathyroidectomy followed by reimplantation of the thyroids. It was mentioned earlier in this paper that several investigators (Vincent, '22, and Vincent and Jolly, '05, '06) hold the view that the symptoms resulting from parathyroid removal in the cat may in part be due to thyroid injury and that injury or destruction of the gland plays an etiological rôle in the production of tetany.

In order to obtain definite evidence that the thyroid of the cat is not concerned in the causation of tetany, the thyro-parathyroid apparatus was removed from three animals and the thyroids reimplanted. Immediately after extirpation the glands were placed in a mixture of warm, sterile Ringer's solution and blood serum of the animal from which the glands were taken. All parathyroids were dissected away under the microscope and the thyroid lobes freed from their investing membranes transplanted into the sternohyoid muscles. The complete data concerning two cats so treated is given in the protocols.

Protocol cat 11

July 30. Right external and internal parathyroids removed without disturbing the right thyroid lobe as parathyroids were superficial; the left thyroid with its adhering parathyroids removed, the latter dissected away and the thyroid reimplanted. Adult female; weight, 2180 grams.

August 1, 2. Vomited several times; refused food; greatly depressed but shows no tremors or paw shaking.

August 3. Cat found dead; weight, 2030 grams; large quantity vomited material in cage. Autopsy showed no parathyroids.

Protocol cat 13

July 31. Thyroparathyroidectomy followed by reimplantation of thyroid lobes into sterno-hyoid muscle; female, weight, 1690 grams.

August 1 to 6. No symptoms; wound healed per primum.

August 7. Tetany symptoms; violent tremors of head and back muscles; marked extensor spasms of hind legs.

August 8, 9. Very stiff in hind legs—walks as if on stilts; typical paw shaking.

August 10. 'Tetany eye-sign'; marked tremors; fibrillar twitching of body muscles and muscular incoordination; refuses food.

August 11. All symptoms increased; refuses food; prostrate; continuous muscle tremors and twitchings.

August 12. Died in epileptiform fit judging by condition of cage. Autopsy showed no parathyroids or accessory glands; the thyroid grafts were in excellent condition.

The experiments just recorded, although not as extensive as could be desired, clearly indicate that the presence of thyroid tissue within the organism fails to exert any beneficial influence upon the symptom complex following parathyroid extirpation. The protocols show that one animal died of violent tetany, whereas the other died of depression, both types of reaction characterizing parathyroid insufficiency in the cat.

It will be noted that in one animal, cat 11, the right thyroid lobe was not injured in any way nor was there any interference with the blood supply. Whether or not the grafted tissue would have remained viable for any considerable length of time our experiments do not show; but at any rate, even though such autoplastic transplants were slowly undergoing resorption, considerable thyroid hormone should have been obtained as a result of this process and there should not be thyroid insufficiency.

The parathyroids of another cat were removed and the thyroid lobes reimplanted, but the animal failed to show any but transient tetany symptoms and completely recovered within a few days. The animal was killed for examination twenty-nine days later, and autopsy revealed the presence of three small accessory parathyroid glands embedded in the

upper portion of the thoracic thymus. The failure of this cat to succumb to tetany was not due to any influence exerted by the thyroid implants, but to accessory parathyroids. The protocol of cat 7 (p. 500) offers convincing evidence of the correctness of this assertion.

A series of autoplastic transplantations of the parathyroids whereby tetany was prevented followed later by reextirpation of the implanted parathyroids and development of fatal tetany, show beyond reasonable doubt that the thyroid of the cat plays no part in inducing tetany in this animal. These experiments will be given in detail in a separate communication, dealing with the subject of auto- and homoplastic parathyroid transplantations.

Transient tetany. Ten operated animals, in which all four parathyroids had been identified and removed, developed definite tetany symptoms shortly after the operation, but later completely recovered and remained normal throughout the period of observation. Generally in such cats, tetany is benign in character and violent symptoms do not appear, the tetany consisting of slight tremors, fibrillar twitching, paw-shaking, and eye signs. However, two cases were observed in which very violent tetany developed, and while these were regarded as fatal cases, to our great surprise both animals recovered.

Some of the earlier investigators (Vincent and Jolly) have recorded similar observations on cats deprived of the usual four parathyroids and regarded such cases as indicating that the cat can exist without parathyroids, i.e., that the latter are not absolutely essential for life in some individuals of this species.

As a result of our observations on animals exhibiting transient tetany, we are led to an entirely different interpretation of the phenomenon. In our opinion, transient tetany is due to the sudden deprivation of the organism of the major portion of its parathyroid tissue, thus inducing the tetany syndrome, before the accessory glands, usually if not invariably present in such cases, can assume the added

secretory burden thrust upon them. In seven of our ten cases of transient tetany accessory parathyroid tissue was found; the remaining three animals, though carefully dissected, showed no accessory glands. However, despite the failure to find them in these animals, it is very probable that minute nodules of parathyroid tissue were present. Absolute certainty that no such tissue was present would necessitate serial sections of the entire cervical region and thoracic thymus. This was not feasible. Our experience leads us to believe that some animals dying of tetany probably have small amounts of accessory parathyroid tissue, and a fatal issue could be avoided if the sudden onset of violent symptoms could have been delayed a few days, thus giving the accessory glands time to compensate the physiological deficiency due to ablation of the main mass of parathyroids. Protocol of a typical case follows:

Protocol cat 16

August 3. Thyroparathyroidectomy. Adult male; weight, 3890 grams. All four glands identified.

August 4. Tremors, fibrillar twitching of back muscles; paw shaking and extensor spasms of hind legs; animal stiff legged; refuses food.

August 5. Tremors less marked; refuses food.

August 6. Exhibits cerebellar symptoms—due possibly to weakness from lack of food; legs stiff; hallucinations.

August 9, 10. Walks unsteadily; legs stiff and spraddled; balances with difficulty; eats liver and drinks milk.

August 11. Condition better; conjunctivitis disappeared; paw shaking noted; occasional extensor spasm of hind legs.

August 12 to 17. Animal recovering; sleeps most of time; eats heartily.

August 20. Completely recovered; weight, 3970 grams. Reoperated; large accessory parathyroid, 5 mm. long, found cephalic portion of the thoracic thymus. Animal died from overdose of ether.

It is unfortunate that this cat could not have been kept alive for several days after removal of the accessory. In all probability, however, it would have succumbed to violent tetany. The protocol of cat 7 (p. 500) is the record of a

similar case except that the animal survived the reoperation and died of tetany following removal of the accessory glands.

Mention was made early in this section of two cats which developed very violent tetany following parathyroid removal, but subsequently recovered. Vincent and Jolly ('05) mention a similar case. Such cases are unusual and therefore of much interest. The protocol of one such animal is given below.

Protocol cat 31

August 21. Thyroparathyroidectomy. Adult female; weight, 3090 grams. All parathyroids identified. The cervical thymus was also extirpated.

August 22. Slight tremors of body and head muscles; paw shaking.

August 23. Violent tetany; prostrate; cannot stand; marked tremors and fibrillar twitching of muscles; marked extensor spasms of fore and hind legs; refuses food.

August 24. Prostrate; mews continually; marked extensor spasms of legs; tremors of body muscles; refuses food.

August 25. Much better; few tremors; animal walks unsteadily.

August 26. Symptoms abating; drinks milk but refuses meat.

August 27 to 30. All symptoms except weakness disappeared. Weight, 2270 grams.

This cat was later killed and the material from the thoracic thymus saved for sectioning. During the ten days of the experiment the cat lost 820 grams in weight. At the present writing the preserved material has not been sectioned, owing to the pressure of other work.[2] In view of other data presented in this section, it seems probable this animal had accessory parathyroids in the thoracic thymus. Paton and Findlay ('17) have figured an accessory parathyroid of a cat in such a location, and Shapiro and Jaffee ('23) report that 12 per cent of cats have accessories in the thoracic thymus. We have noted one case, an animal dying of tetany, in which a small accessory parathyroid was found in the thoracic thymus (see section on accessory glands, p. 498).

Sex, age, pregnancy, diet, and shock as factors influencing the onset of tetany. 1. Sex. The sixty-two operated animals

[2] Since this was written the material has been sectioned and accessory parathyroid tissue found in the cephalic portion of the thoracic thymus.

of our series were about equally divided as to sex. There were twenty-nine males and thirty-three females. So far as we have been able to observe, sex has no effect in hastening, delaying, or modifying in any way the symptom complex following parathyroid extirpation. So far as we are aware, no one has reported any differences between the sexes in reaction to parathyroid removal in any species.

2. Age. In contrast to sex, the age of the animal plays an important rôle in the symtomatology of parathyroid insufficiency. Young kittens react differently from older animals. Of ten young animals (kittens weighing less than 800 grams and some as little as 300 grams), six died in depression, two succumbed to violent tetany, and one developed only transient symptoms due to the proved presence of accessory glands.

The older animals showed much less depression; generally in older animals periods of depression alternated with periods of tetany, the cats dying in coma or convulsions. With the exception of young kittens, then, the great preponderance of our fatal cases were due to violent tetany. In dogs the situation seems to be different, because several investigators have stated that depression is of equal importance with tetany in causing the death of these animals following parathyroid removal.

3. Pregnancy. Carlson ('13) demonstrated by a series of parathyroid extirpations upon pregnant dogs and cats that pregnancy is a factor which must be taken into account after parathyroid operations, because in certain forms it exerts a marked influence upon the resulting symptoms. He found that pregnancy in dogs tends to aggravate the tetany symptoms and the fatal termination is more rapid than is the case with non-pregnant animals. In the cat, however, the fatal issue was not hastened, but the number of animals dying of depression was abnormally high.

The number of cases in our series is too small to be of considerable value. Of five animals in late pregnancy, two died of tetany following operation, one had accessory parathyroid tissue, as shown by autopsy, and did not develop

symptoms, and the remaining two were autoplastically transplanted with parathyroid tissue immediately following gland removal, and hence are without significance for the problem under discussion. The two cats in late pregnancy did not abort despite extremely violent symptoms (repeated spasm and epileptiform convulsions).

4. Diet. It has long been established that the nature of the diet is of very considerable importance in conditions resulting from parathyroid insufficiency. The effect of a meat diet on parathyroidectomized dogs is common knowledge, and the recent important work of Dragstedt and his collaborators shows very clearly the potency of certain dietary factors in controlling tetany. We have no additional information concerning the effects of diet. Our animals were fed, both before and after operation, on a uniform diet consisting entirely of milk and beef liver.

5. Shock. We have had occasion to observe many times that operated animals, suffering from depression, but showing no tremors or twitchings, can quickly be thrown into violent tetany by shocks or by rough handling. Sometimes merely tapping on the metal bars of the cage with a key is sufficient to induce spasms in animals which had previously shown no violent symptoms. Recently Dragstedt has listed temperature as a predisposing factor to tetany symptoms in operated dogs.

Accessory glands. There can be little doubt that the presence of accessory parathyroids in the cat is responsible for much of the conflicting data found in the literature concerning the results of parathyroidectomy in this species. By accessory glands, we refer to aggregations of parathyroid tissue other than the usual four glands which have no intimate anatomical relation to the thyroid lobes. Supernumerary glands in close proximity to the usual four parathyroids occur fairly frequently. Several such bodies are shown schematically in figure 6. In reality, they, too, should be classified as accessory glands, but inasmuch as they would be removed at a thyro-parathyroidectomy, the term accessory has been

reserved for those glands which would not normally be included at such an operation. Such aberrantly situated glands have been found to occur in a high percentage of cats. To the best of our knowledge, no other mammal has been reported in which accessory parathyroid glands are of such frequent occurrence.

TABLE 3

NUMBER OF ANIMALS EXAMINED	NORMAL NUMBER AND RELATION TO THYROID	MORE THAN 4 GLANDS PRESENT IN NORMAL POSITION	ACCESSORY GLANDS
77[1]	45 58.44 per cent	5 6.49 per cent	27 35 per cent

[1] Since this was written, one of the authors (W. W. S.) removed the parathyroids from forty-five additional cats, making a total of 107 operated animals examined, not including those killed for anatomical purposes. The per cent of cats possessing accessory glands is approximately 35 per cent when all cases are considered.

Only those cases are included in the table in which accessory tissue was actually found at necropsy. Animals dying of tetania parathyropriva were seldom examined as carefully for accessory glands as operated individuals which remained normal, and many were not examined at all. It was assumed that cases terminating fatally did not possess accessory tissue or, at any rate, but very little. The admission is freely made, however, that such an assumption is purely gratuitous. Recently, one of the writers in the course of a postmortem examination of an animal dying of tetany found a small nodule of suspicious-looking tissue embedded in the cephalic portion of the thoracic thymus; microscopic examination of sectioned material confirmed the suspicion that the tissue was an accessory parathyroid.

Shapiro and Jaffee ('23) report that accessories may be demonstrated in the thoracic thymus of cats which died of parathyroid insufficiency, showing tetany or depression or both. The basis for this statement is not clear from a perusal of their paper, as no data are presented. Presumably, they

mean that the cats in which they found accessories in thoracic thymus had died of parathyroid insufficiency, although no data for such statement appear in their paper. The only remark made in this connection was that "Forty-two thymuses obtained in the routine autopsies in the laboratory . . . were used." The cause of death is not mentioned.

Harvier and Morel ('09) state that accessory tissue is present in 50 per cent of cats, but the number of animals upon which this conclusion is apparently based consists of less than five. One of the writers operated upon a series of eight cats within four days, in all of which the normal four parathyroids were found, but only two cases were fatal, the remainder showed merely transient tetany. At autopsy accessory parathyroid tissue was found to be present in three animals. The remaining three showed nothing despite careful dissection of the cervical region and thoracic thymus; however, as we have stated before, the failure to find accessory glands does not necessarily prove their absence. Anyone who has searched for accessory parathyroids in an operated animal at autopsy will readily appreciate the difficulties involved and the possibilities of overlooking them.

Marine ('14) states that about 4 to 6 per cent of dogs possess accessory glands, consequently this animal is much more favorable for experimental purposes than the cat. A survey of the literature on parathyroid extirpation in the dog shows much more uniform and consistent results than on cats. Material in which one animal in three has accessory glands cannot be expected to give very consistent results unless a very large number of animals is used. The surprising thing is not that the results of work on the cat have been conflicting, but rather that investigators such as Vessale and Generali, and Welsh were able to secure the consistent results which they did. The rather small number of cases on which the conclusions of these workers were based indicates that they were exceptionally fortunate in their chance choice of cats.

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The work of Vincent and Jolly ('05) shows about the same ratio of fatal cases to the number of operated animals as our own; they report that out of eighteen operated cats twelve died and six lived. However, they were confident that all parathyroid tissue had been removed in the six surviving animals.

Paton and Findlay ('17) have reported a case in which the cat lived following what presumably was total removal of all parathyroid tissue, as they were unable to find any at autopsy. They do not claim, however, that failure to find accessory tissue is proof of its absence.

The physiological importance of accessory parathyroids is very great, because very minute bits of tissue suffice to keep the animal in normal condition, as shown by the following experiment:

Protocol cat 7

July 24. Thyroparathyroidectomy. Female, weight, 1500 grams. All glands identified.

July 25 to August 11. No symptoms.

August 12. Reoperated. All cervical fat and thymus removed. The site of the old ligatures removed. No parathyroid tissue found.

August 13 to 20. No symptoms.

August 20. Weight, 1190 grams; reoperated; a portion of the thoracic thymus retracted from the superior mediastinum and removed. In this mass were found three minute parathyroid glands.

August 22. Violent body tremors, paw shaking, with epileptiform convulsions; 'tetany eye-sign' present.

August 23. Dying of tetany; violent muscle spasms; prostrate.

August 24. Dead; weight, 1040 grams.

Operated animals possessing accessory glands usually show an increase in weight, unless the general health of the individual is affected by untoward conditions not directly referable to gland removal, such as pneumonia, colds, severe conjunctivitis or infection. On the other hand, operated animals in which (presumably) all parathyroid tissue has been removed show a rapid decrease in weight as shown in table 1. The weight increase in animals possessing accessory parathyroids naturally varies according to the age of the animal

and duration of the experiment. The fact that for several weeks following removal of thyroids and parathyroids (except accessories) cats gain in weight is attributable, in part at least, to the liberal amount of food given and to decrease in the catabolic activities of the organism due to loss of the thyroid. Table 4 shows the weight gains in eight typical cases where accessory parathyroid tissue was found.

Summarizing the results of the foregoing observations on accessory glands, it is evident: 1) that accessory parathyroids

TABLE 4

CASE NO.	DURATION OF EXPERIMENT	WEIGHT		INCREASE
		Beginning	End	
	days	*grams*	*grams*	*per cent*
2 (adult)	12	2100	2150	2.38
5 (adult)	28	1260	1540	22.22[1]
12 (adult)	29	1720	2000	16.12
16 (adult)	19	3890	3970	2.0
32 (adult)	16	3720	3800	2.15
33 (kitten)	13	650	705	8.45
36 (kitten)	11	800	853	6.6
39 (adult)	15	2435	2478	1.7

[1] This animal had been autoplastically transplanted with two external parathyroids at the time of operation.

occur in about 35 per cent of cats; 2) the physiological significance of these structures is identical with that of the normally placed glands; 3) the presence of small amounts of accessory gland tissue in operated cats is sufficient to prevent any untoward symptoms; 4) reoperation and removal of all accessory glands causes fatal tetany; 5) accessories may be present even in cases terminating fatally.

SUMMARY AND CONCLUSION

1. A study of the thyro-parathyroid apparatus of the cat shows that parathyroids are variable in size, number, and location, the internal parathyroids varying more than the externals (cf. figs. 1 and 6, p. 507).

2. Injection methods were correlated with microscopic examination of fresh material in order to determine the variations of blood supply and the number, size, and location of the parathyroids.

3. The importance of the removal of cervical fat and all obtainable thymus in order to secure complete parathyroidectomy in the cat has been emphasized by these studies.

4. Extirpation is preferable to cautery in removing the parathyroids because of the nature of the field of operation.

5. Infections are infrequent, and when they do occur are easily controlled by irrigation of the field with Dakin's solution.

6. Complete thyro-parathyroidectomy in the cat results in death from one of two typical syndromes, tetany or depression.

7. When the thyroid lobes are removed, death does not ensue if one parathyroid is left with undisturbed blood supply.

8. The presence of thyroid tissue, either by transplantation or by the extirpation of the parathyroids leaving the thyroid relatively uninjured, will not prevent death after complete parathyroidectomy.

9. Transient tetany in the cat has been proved in most cases to be associated with the presence of accessory parathyroid tissue. This has been demonstrated in seven of ten cases of transient tetany.

10. During the syndrome resulting from parathyroid extirpation in the cat part of the conjunctive (nictitating membrane) is drawn laterally over the surface of the eye simulating external strabismus. This has been termed the 'tetany eye sign.'

11. The sex of the cat apparently plays no part in the ensuing symptomatology.

12. Age has a bearing upon the syndrome for most of the unmixed cases of depression following parathyroid removal in young kittens.

13. The data secured upon the effects of pregnancy were too few to be significant, but apparently show that pregnancy has no effect upon the symptoms.

14. Shocks are sometimes a factor in bringing about tetany. This is particularly true in transient cases.

15. Accessory glands occur in about 35 per cent of cats, (table 3, p. 498). When accessory glands are present, the animal generally gains in weight after the operation (table 4, p. 501).

16. The accessory glands possess functions identical with those of the normally placed glands as shown, 1) by their presence in cats which have shown no symptoms after a supposedly complete thyro-parathyroidectomy (table 1, p. 482) and, 2) by the rapid appearance of symptoms and fatal termination after their removal (protocol cat 7, p. 500).

17. Accessory parathyroids are infrequently found in cats which have died of tetany.

The only logical conclusion which can be derived from the facts given above is that in the cat, under normal conditions of life and diet, complete parathyroidectomy is invariably fatal.

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PLATE 1

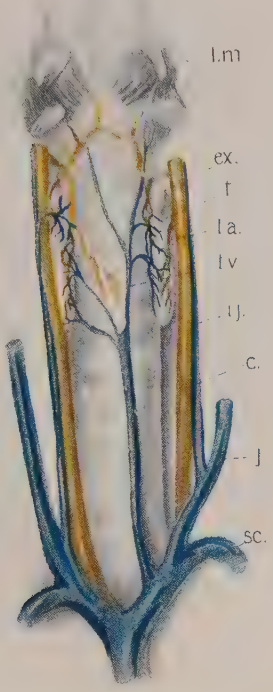
EXPLANATION OF FIGURES

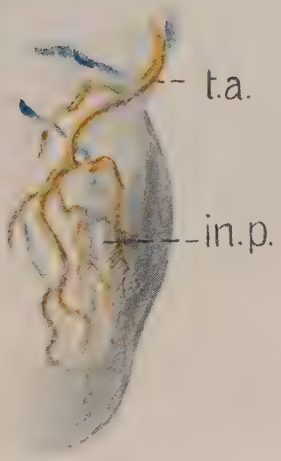
1 Ventral view of the thyro-parathyroid apparatus of the cat. *ex.*, external parathyroid; *t.*, thyroid lobe; *j.*, external jugular; *c.*, carotid artery; *t.v.*, thyroid vein; *t.a.*, thyroid artery; *l.m.*, laryngeal muscles; *i.j.*, internal jugular; *s.c.*, subclavian vein.

2 Thyroid lobe of the cat viewed from the tracheal surface. *in.p.*, internal parathyroid; *t.a.*, thyroid artery. The glands have been dissected free from their investing membranes.

3 Ventral view of the thyroid lobe, showing its relation to the cervical fat and thymus. *t.*, thyroid lobe; *ex.*, external parathyroid; *c.*, cervical fat and thymus tissue.

1





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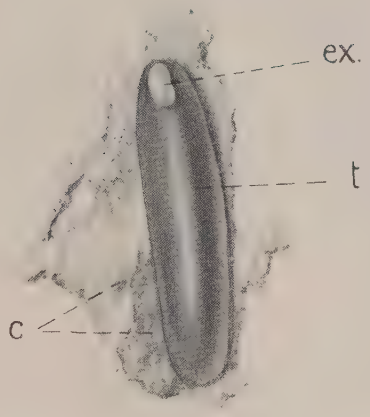
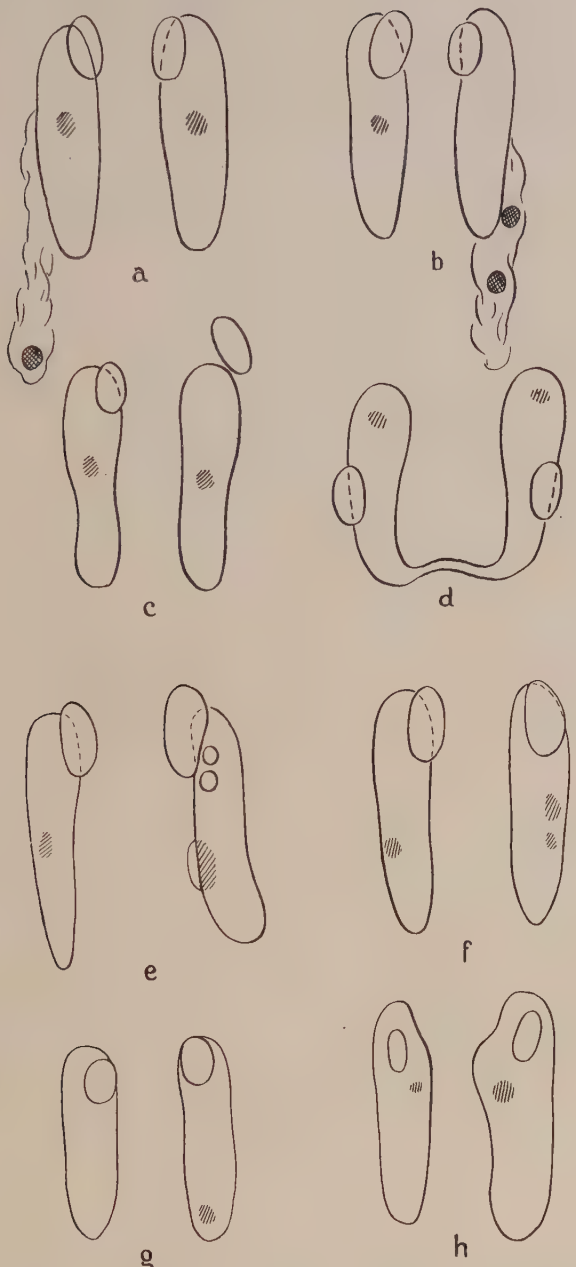


3

4 Section through the external parathyroid gland showing the blood and lymph supply.

5 The 'eye sign' of parathyroid insufficiency in cats. A and B, parathyroidectomized animals; C, normal eye. In A and B the nictitating membrane is drawn over the nasal angle of the eye.



6 Some of the commoner variations in number and position of the parathyroids. The thyroid lobes and external parathyroids are outlined; the internal glands are indicated by parallel lines, accessories occurring in the cervical fat and thymus are cross-hatched.

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine,
1925, xxii, pp. 333-334

162 (2685)

The relation of contractile and food vacuoles to rhythms in Paramecium.

By W. BYERS UNGER. (Introduced by L. L. Woodruff).

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Experiments were undertaken to determine the relation between metabolic activity, as indicated by the rate of contractile vacuole pulsations and the size and number of food vacuoles formed, and the occurrence of rhythms in the division rate. The animals studied were pedigree races of *Paramecium aurelia* and *Paramecium calkinsi*. Two cultures of each species were studied for 115 days under constant culture conditions. Observations were made daily and averages computed for the per diem division rate, food vacuole number and size, and the pulsation rate of the anterior and posterior contractile vacuoles.

In *Paramecium aurelia*,[1] depressions in the rate of contractile vacuole pulsation are, in general, synchronous with the low points of the rhythms in the division rate and with the occurrence of endomixis. The posterior contractile vacuole pulsates more rapidly than the anterior. The variations in the number of food vacuoles formed are, in general, synchronous with the rhythms in the division rate and the fluctuations in the rate of pulsation of the contractile vacuoles.

Although endomixis does not occur in *Paramecium calkinsi*,[2] this species exhibits definite fluctuations in the rates of the two

[1] Woodruff, L. L., *Biol. Bull.*, 1917, xxxiii, 51.

[2] Spencer, H., *J. Morph. and Physiol.*, 1924, xxxix, 548.

contractile vacuoles which corresponds to the rhythms in division rate. The anterior and posterior contractile vacuoles show no significant difference in rate. As in *P. aurelia,* the fluctuations in the number of food vacuoles formed in *P. calkinsi* is synchronous with the rhythms in reproductive activity and the fluctuations in contractile vacuole pulsations.

Paramecium calkinsi forms more food vacuoles than *Paramecium aurelia,* though the size of the vacuoles is smaller in the former. The rate of pulsation of the contractile vacuoles is faster in *P. aurelia* than in *P. calkinsi;* a difference probably correlated with the smaller size of *P. calkinsi.*

The fluctuations in the several phenomena observed are not caused by endomixis since rhythms are present in *P. calkinsi* (where endomixis does not occur) as well as in *P. aurelia* (where endomixis does occur). Apparently the rhythms in *P. aurelia* are accentuated by endomixis since the changes in the rate of the contractile vacuoles, in particular, are more abrupt and extend over a greater range than those of *P. calkinsi.*

In brief, fluctuations in nutrition and excretion, as indicated by food vacuole formation and contractile vacuole pulsation, are shown to be coincident with, and probably causally related to, the well-established rhythms in the reproductive activity of Paramecium.

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 41, No. 4, April, 1925

THE DEVELOPMENT OF THE BALANCER IN AMBLYSTOMA, STUDIED BY THE METHOD OF TRANSPLANTATION AND IN RELATION TO THE CONNECTIVE-TISSUE PROBLEM

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SIXTY-TWO FIGURES

During the course of an investigation of the development of the gills in Amblystoma, involving transplantations of the ectoderm of the head,[1] it often occurred that the balancer of the grafted embryos developed in an abnormal place. This in itself was not new, for in 1907 Bell had published an interesting paper in which it was shown, by means of grafting, that the ectoderm initiates the development of this organ. Nevertheless, many questions of interest remained unsettled, and the present study is an attempt to solve some of these.

Our knowledge of the balancer is so fragmentary and the literature so widely scattered, that it has seemed desirable to bring together, as far as possible, what is known of the subject, and to try to fill the gaps in our knowledge of the normal structure and development of this enigmatic organ before proceeding to the consideration of the experiments.

The balancers are a pair of slender rod-like appendages which project from the side of the head a little behind and below the eyes, and which in Amblystoma punctatum, as Clarke ('79) first pointed out, serve as props to hold the head off the bottom and to prevent the larva from falling over on its side, until the fore-legs develop and assume that office. The balancers develop synchronously with the external

[1] Cf. Harrison, '21.

gills and drop off about the time the yolk is completely resorbed.

Only certain species of salamander embryos have balancers. Rusconi (1821) and Van Bambeke ('80) described them in Triton, the latter calling them 'crochets de Rusconi.' Altogether they have been noted by various authors in four species of this genus (helveticus, alpestris, taeniatus, and cristatus). They occur in Salamandrina perspicillata according to Lessona ('74), Camerano ('85), and Clemens ('95),[2] in Pleurodeles waltlii (Vaillant, '80),[3] in Isodactylium schrenkii (Hynobius keyserlingii) (Shitkov, '95), in Hynobius naevius, where, according to Tago ('07), as quoted by Dunn ('23), they are said to be double, in Diemyctylus viridescens (Bell, '07), and in several species of Amblystoma, including A. punctatum (Clarke, '79), A. opacum (Latta, '19), A. jeffersonianum, and A. microstomum.[4]

The absence of balancers in the axolotl (A. tigrinum) was noted by Van Bambeke, who, however, thought that they appeared in rudimentary form at an early stage, only to fail in their further development. Maurer ('88, p. 196) was of the opinion that they might occur sporadically in the axolotl embryo, and mentions that out of sixty cases he found one in which they were present, though less developed than in Triton. No trace of the organ has been found in the material used in the present investigation beyond the initial thickening and slight bulging of the ectoderm, which is heavily pigmented at that point (p. 365). Part of this material was collected in the vicinity of Lake Forest, Illinois, and part laid

[2] The presence of the balancer in Salamandrina is shown in figure 14 of Camerano's paper and in figure 22 of Clemens, which latter is taken from a drawing by Wiedersheim. No mention of the organ is made in the text of either paper in connection with this species.

[3] This paper, which is very brief, gives no description of the development of the balancer. It is merely mentioned as follows (p. 128): "Les crochets d'adhérence disparaissent au treizième jour à partir de l'éclosion: les jeunes têtards possèdent à cette époque un bras tridactyle."

[4] Their occurrence in the two last-named species has been observed by Dr. Henry Laurens and myself. The eggs of A. microstomum were identified as such by Prof. G. E. Coghill, who kindly sent them from Lawrence, Kansas.

in the laboratory by some very large axolotls, said to have been collected in North Dakota. Other material from Lawrence, Kas.; Minneapolis, Minn.; Chicago, Ill., and Urbana, Ill., also failed to show balancers. On the other hand, Nicholas ('24) has recently found that in a certain strain of A. tigrinum, said to have come from Wisconsin or possibly from North Dakota, a large proportion of the embryos (between 80 and 90 per cent) have rudimentary but quite distinct balancers, which appear in the usual position in stages 36 to 40 and then disappear, never developing beyond a nodule or short process. Sections show that the epithelium becomes columnar, as in A. punctatum, that it evaginates forming a pocket filled with mesenchyme, but that the basement membrane is not formed.[5]

Figures of the embryos and larvae of other species of tailed amphibians given by various authors indicate that the balancer is absent in Proteus anguineus (Wiedersheim, '90), Necturus maculosus (Eycleshymer and Wilson, '10), Cryptobranchus allegheniensis (B. Smith, '12), Megalobatrachus (Cryptobranchus) japonicus (Kerbert, '04, and Ischikawa, '08), Spelerpes bislineatus and Desmognathus fusca (Wilder, '99), Plethodon cinereus erythronotus (Piersol, '09), Hemidactylium scutatum (Bishop, '19), Euproctus asper (Despax, '23) and Autodax lugubris (Ritter and Miller, '99).[6] It is also probably absent in Amphiuma(Hay, '88), though this

[5] I am indebted to Dr. J. S. Nicholas, of the University of Pittsburgh, for calling my attention to this discovery and for permission to use the above information in advance of its publication.

[6] Generic and specific names are here given as used by the authors cited. One who consults the recent check list by Stejneger and Barbour ('17) will find there that many of the names are different, as for instance, Notopthalmus for Diemyctylus, Eurycea for Spelerpes, and Ambystoma for Amblystoma (A. maculatum for A. punctatum). No attempt has been made here to confirm this revision, and it may be assumed that the new names are in strict accordance with the rules of the Nomenclature Commission. I wish to protest, however, against the practice of unearthing old and forgotten names merely to conform to an arbitrary rule, as has been done in the case of Amblystoma, which has figured so largely in embryological and experimental work. Instead of rejoicing that the obsolete and meaningless form of the name given by Tschudi (probably through oversight) has been lost, 'Ambystoma' is dragged again to the light

is not absolutely certain, since in this form only one sufficiently early developmental stage is known. None of this group of writers refer explicitly to the balancer except Piersol, who says (op.cit., p. 484) "Plethodon has neither the balancers nor the adhesive discs common among the other amphibian larvae." In the gymonophion embryo there is likewise no indication of the presence of balancers, as shown by Brauer's ('99) figures of Hypogeophis[7] and Göldi's ('99) figures (only one stage) of Siphonops.

In the foregoing enumeration of species the balancer is found either to occur in its full development or not to be present at all except in those strains of A. tigrinum in which it is rudimentary. Salamandra appears to be the only genus in which it regularly occurs in rudimentary form and is resorbed very early. It apparently escaped the notice of Rusconi ('54), whose authority Clemens ('95) accepts. Wunderer ('10), however, in a very thorough study of the life-history of that remarkable form, S. atra, showed that a distinct outgrowth appears in the same place and at the same time as the balancer in other forms (e.g., stage 35 in Amblystoma punctatum), but persists only until a stage corresponding to ca. 39 in Amblystoma is reached, at which time it is already reduced to a small knob. Also in S.

of day only to bring confusion and vexation with it. Equally unfortunate is the changing of the specific name of the spotted salamander from punctatum to maculatum. The grounds for this change are quoted from Stejneger ('02) and need no comment. "Ambystoma maculatum is usually known as A. punctatum (Linnaeus), being the Lacerta punctata of the twelfth edition of the Systema Naturae. This name was preoccupied by Linnaeus himself in the tenth edition for a true lizard, now known as Riopa punctata. Its use is therefore precluded. The next name is Bechstein's Salamandra palustris, but this name was also preoccupied the year previously by Schneider for the female yellow-spotted Salamander of Europe. Shaw's Lacerta maculata is the next name in time, and is apparently not preoccupied. These names are absolutely equivalent, being all based on Catesby's 'Car. 3, p. 10, t. 10, f. 10.'" As Scott (Science, vol. 44, p. 310) remarks: 'Ambystoma, then, it seems, has no right except the right of having started wrong.'

[7] Brauer describes short processes which appear on the mandibular and hyoid arches about the time of appearance of the external gills. The first is of very transitory nature and there is nothing to indicate that it is a rudimentary balancer.

maculosa, Wunderer found a small balancer nodule in embryos of the stages in question.

According to the above, the occurrence of balancers is limited to the embryos and early larvae of three families of urodeles, the Amblystomidae, the Salamandridae, and the Hynobiidae which, according to the classification of Dunn ('22), belong to two distinct superfamilies, the Salamandroidea and the Cryptobranchoidea. However, the organ cannot be regarded as a diagnostic character, since each of these superfamilies includes families, the Plethodontidae and the Cryptobranchidae, respectively, in which the balancer does not occur. Likewise, it is usually lacking in at least one species of Amblystoma (tigrinum) and is very rudimentary in Salamandra. The occurrence of the balancer, which from the standpoint of taxonomy is apparently sporadic, has been correlated by Noble with the pond habitat of the larvae (see foot note 11, p. 354.

Several divergent views have been held with regard to the homology of the balancer. Spallanzani (1784) first described it as a gill, figuring it erroneously as a tuft of filaments. By a number of recent authors it has also been thought to be equivalent to the external gills, Kingsley ('99) referring it to the hyoid arch, Orr ('88) and Kerr ('19) to the mandibular. Others have coquetted with this view, but it has not been generally held since Maurer ('88) showed that the return blood from the balancer enters the jugular vein and not the aorta, and that the epithelium covering the organ is cuboidal instead of flat. Maurer was, however, rather inclined to think that it may have originally been a mandibular gill.[8] Kingsley ('99) figures a model by Lambert showing the vessels of the balancer and the gills, essentially as described by Maurer, but says that "relations, blood supply and nerves go to show that the fleshy processes (so-

[8] In a later publication Maurer ('02) takes a more decided stand when he says (p. 125): "Dass er keine Kieme ist ergiebt sich aus der Art seiner Einschaltung in den Blutkreislauf. Hautsinnesorgane sind ebenfalls nicht in dem ihn überziehenden Epithel enthalten."

called balancers) of the urodele larvae are the modified gills of the hyoid arch."

On the other hand, the balancers have been likened to the adhesive organs or 'suckers' of the anuran embryo.[9] Thus Rusconi, writing a hundred years ago, distinctly says: "The two former (processes on the side of the head) are not gills, as some authors would have us to believe, but are analogous to the two hooks, which we observe below the head of the embryo of the green frog, by means of which those embryos attach themselves to the leaves of the lentil, and remain suspended." Who the earlier authors were, aside from Spallanzani, I am unable to find out. Rusconi gives a figure showing a Triton larva suspended from a leaf by means of its balancer, much as an anuran tadpole is held by the secretion of its cement organ, and says further on, in describing the movements of the newly hatched larva, that it becomes attached to the body it strikes "by means of its hooks (crochets), the extremities of which are covered with a viscous matter." Van Bambeke ('80) accepts this view of the homology of the balancers, though without giving additional evidence, as also Balfour ('81), who speaks of them as 'stalked suckers' of the same nature as the 'suckers' of anuran amphibians.[10] There are other writers who agree with this view, the most recent being Latta ('19), but as yet no compelling evidence has been adduced in its support.[11]

[9] The terms 'crochets de Rusconi,' 'crochets d'adhérence,' Rusconi'sche Häkchen, 'Uncini di Rusconi,' used to designate the balancer, might be considered appropriate for Triton. For the Amblystoma larva, however, the terms 'balancer,' 'Balanzierstange,' 'Stützorgan,' 'Kieferfortsatz,' seem more fitting. The terms 'stalked sucker,' 'succiatoio,' and others of like etymology are scarcely admissible, even if the homology of the balancer with the cement organ of anurans be accepted, since neither of these structures functions as a sucker.

[10] Both Maurer ('88, p. 196) and Egert ('13, p. 280) erroneously ascribe to Balfour the view that the balancers have a sensory function (Tastorgan).

[11] Dr. G. Kingsley Noble, of the American Museum of Natural History, New York, to whom I am greatly indebted for a critical reading of the present paper in manuscript and for calling attention to several important omissions in the bibliographic references, has kindly permitted me to quote from a letter his own views on the significance and occurrence of the balancer: "I have always found it helpful to compare the urodele balancer directly with the adhesive organs of frogs, especially with that of some of the more specialized species (Hyla). It

does appear superficially, but more so when sectioned, directly homologous with the anuran adhesive organ. In both Urodeles and Anurans these adhesive organs seem fundamental structures—tending to occur in all forms where they can function. In the Anura they are found with a single exception in all aquatic tadpoles and are absent only in those forms which undergo a more or less prolonged existence within the egg capsules. They are naturally absent in those species which lay their eggs on land to develop directly into frogs. Similarly in urodeles, save for two exceptions (A. tigrinum and the Proteidae), the Balancer develops in all forms where it can function. It is present in all pond larvae (hynobiids, salamandrids, ambystomids) and apparently absent in all stream or swift current forms as well as in all terrestrial forms.

"To be sure, only a fraction of all the Urodele larvae have been examined for balancers, but enough forms, I believe, to use the above statement for a working hypothesis. Among the most primitive forms, the Hynobiidae, we find that all the pond larvae studied (Hynobius keyserlingii and H. naevius) have balancers. Dunn ('23) has given a convenient summary. The latter species is said to have 'double balancers' on each side. The mountain brook hynobiid Ranodon sibiricus apparently lacks Balancers (Snitnikov, Ann. Mus. Zool., St. Peterbourg, vol. 18, 1913). The Cryptobranchidae have evolved directly from hynobiids. They are confined to the 'stream-habitat' and in correlation with this habitat lack Balancers. Among the salamandrids (including Pleurodeles) it is only the mountain brook form (Euproctus asper, Despax, '23) which apparently lacks them. The early larvae of the mountain brook ambystomids are not known. All other ambystomids (of those described) save A. tigrinum possess Balancers. The Plethodontidae originated in a mountain brook habitat, as shown by their distribution and organization. (Witness their lungless condition which is found in all those genera—and in only those genera of ambystomids and salamandrids which live near mountain brooks). Further, all plenthodontids with one exception either lay their eggs in mountain streams or on land. It is therefore only 'natural' that they should lack Balancers. Amphiuma has been derived from the salamandrids but as the species lays its eggs on land and the larvae hatch in an advanced condition we would not expect that they should have Balancers. On the other hand, the rudimentary Balancers of Salamandra prove what we already suspected on morphological grounds that Salamandra has been recently derived from some of the more primitive newts (Tylototriton, etc.).

"This leaves only two families of Urodeles to account for. I frankly admit that I am at a loss to explain away the balancerless condition in the two genera included in the Proteidae, unless it be that the relatively large amount of yolk precludes a 'very early' larval life outside the egg capsules. Proteus, as you know, may give birth to its young alive, which speaks well for the yolk when so far as we know the species has no special modifications in either young or adult for intra-oviduct development. The early larva of the last family, Sirenidae, are unknown, but I have recently examined a larval Pseudobranchus with fingers undeveloped and arm not yet rotated. This little fellow was in sad need of a balancer for he could lie only on his side. He exhibited, however, a pigmentary disturbance in the proper position for a balancer and I would not be at all surprised if at an earlier stage he possessed a non-stalked Balancer of the Anuran type."

The homology of the balancer with the tentacular apparatus of the Gymnophiona and of the larva of Xenopus has been advocated by a number of writers, as, for instance, Cope ('89), Boulenger,[12] Bles ('05), Gadow ('09), and Cunningham ('12), notwithstanding the fact that the tentacle of Xenopus is attached near the corner of the mouth in front of the eye, while the urodele balancer is posterior to the eye. The possibility of the balancer having a sensory function like the barbels of certain fishes has also been considered. Other observers (Kingsley, '02; Egert, '13, and Latta, '19) have found, on the other hand, nothing to support this view, and it may be dismissed as without foundation. The balancer does, however, have a nerve.

As will already have been inferred from the foregoing, the function of the balancer has been as much a matter of divergence of opinion as its homology. Rusconi emphasizes the adhesive nature of the appendage in Triton, but none of those who have studied Amblystoma record having seen the embryos of this form hanging by their balancers as Rusconi figures the Triton larva. As Clarke has demonstrated, the organs in Amblystoma are used as props which keep the head off the bottom. Both Egert ('13) and Latta ('19) confirm this, showing that when the balancers are removed the larva comes to lie on its side, and that on a soft bottom the head and gills may often be partly buried. This is the view adopted also by Gadow and by Cunningham, but the former considers it not unlikely that they may acquire a sensory function in addition. Oddly enough, both state that the balancer is movable, although this is not the case except passively. This idea is probably based upon Orr's statement[13] that there is a bundle of muscle fibers extending from the pterygoid muscle into the base of the balancer, and also another band of undifferentiated muscular elements running from the end of the articular process. In a very careful study of a large number of cases, I have been unable to detect

[12] In a footnote to a paper by Leslie ('90, p. 70).

[13] Op. cit. p. 317.

any muscle fibers whatever in the balancer. Orr's figures, moreover, are not convincing, and it is possible that the nerve, which is found in every case, has been mistaken for a bundle of muscle.

The circulation in the balancer has been an important factor in the interpretation of the significance of the organ. Clarke described the blood coursing in its vessels, which consist of a central artery and vein in which the amount of blood circulating grows less and less as the time for shedding the organ is approached. It stops altogether before the latter event. In a paper on the gills and their blood vessels in the amphibians, Maurer ('88) describes the development of the balancer (termed 'Kieferfortsatz') and shows that it is supplied very early in its development by a branch of the hyomandibular artery, which runs dorsally past its base, much as the gills are supplied by branches of the proper aortic arches. The return vein of the balancer, however, unlike the efferent branchial vessels, does not empty into the dorsal aorta, but into the jugular vein.

The foregoing will suffice to show the fragmentary nature of what is known of the balancer. In fact, there seem to be but three papers which treat primarily of this organ.

The first and most original of these is the one by Bell ('07), who showed, by means of transplantation experiments on Diemyctylus embryos, that it is a small area or ectoderm lying on the mandibular arch that determines whether a balancer shall develop. Bell also described the normal development of the organ as seen in sections and was the first to note and interpret correctly the peculiar basement membrane characteristic of it.

The paper by Egert ('13) deals principally with the homologies of the balancer, which is called by him the 'Stützorgan,' because of its function in propping up the head. Egert points out that the balancer sticks readily to solid objects as shown by the readiness with which the embryo may be drawn along by a needle applied to the tip. No mention is made of a special secretion, and the thick basement

membrane, which gives the organ its rigidity, is overlooked, this property being supposed by him to be due to the turgor produced by the blood stream. Although Egert uses the term 'festhaften' with reference to the balancer, he does not mention any relation to the adhesive organs of the anurans. In conclusion, he thinks it might be regarded as a modified gill, if it can be shown to belong to the hyoid arch, but hardly so if shown to belong to the mandibular.

The latest paper is by Latta ('19), who accepts Clarke's views that the organ functions as a prop. Latta pays especial attention to the basement membrane, but he regards it as a dermal bone, without, however, giving the ground for this interpretation. He points out that the basal portion of this structure is left embedded in the underlying tissue after the balancer is shed, but takes no part in the formation of any permanent bony structure.

In the following pages the normal structure and development of the balancer are first considered. This part of the study is based upon both the living or fresh material and embryos preserved, for the most part, in the usual corrosive sublimate-acetic mixture and afterward stained in Mallory's connective-tissue stain. This stain, though capricious, gives pictures of surprising beauty and clearness when properly applied, and much that has been seen with the aid of this method would have remained obscure in sections stained in haematoxylin followed by one of the usual plasma stains. It soon became evident that the basement membrane was one of the most important features of the balancer. Further study made it clear that the development of this structure afforded a key to the solution of the problem of the histogenesis of connective tissue, for it is seen to develop out of an intercellular ground-substance without direct participation of mesenchyme cells. The nature of the basement membrane was studied by means of the chemical tests usually applied to the connective tissues (Mall, '96), whereby it was shown to be of a reticular nature and not osseous. The presence of a nerve as a constant constituent of the organ

was also established, no previous observation to this effect having been, to my knowledge, recorded. In confirmation of the observation, first recorded by Orr and afterward extended by Winslow ('98), a distinct articular cup or process on the palatoquadrate cartilage was found. This serves for the articulation of the basement membrane and disappears, as Winslow also noted, shortly after the appendage is shed.

The experiments were made for the purpose of analyzing the factors involved in the development of the balancer. They deal with the respective rôles played by the tissues that enter into its composition, with the specificity of the reactions between these tissues and with the time factor of the developmental processes.[14]

THE NORMAL STRUCTURE AND DEVELOPMENT OF THE BALANCER

External features as seen in the living or freshly treated specimens

The following observations were made upon embryos and larvae of Amblystoma punctatum (Linn.), the species used in most of the experiments. The balancer first appears on the exterior of the embryo, in stage 34 (fig. 1) as a faint rounded nodule upon the mandibular arch lying ventroposterior to the eye. It becomes much more distinct in the next stage, at which time there are no gill sprouts, though the branchial swelling when viewed from above is clearly divided into three ridges. A little later (stage 36) the balancer which is now considerably elongated 'points' distinctly ventroposteriorly. The three external gills have begun to sprout to about the same extent as the balancer. Stage 37 (fig. 2) finds the balancer a cylindrical projection, about 0.25 mm. long with its tip rounded off, converging toward the first gill which

[14] For the illustrations I am much indebted to Mr. H. D. Rhynedance, who drew most of the text figures, and to Miss J. E. Lovett and Miss L. Krause, who drew the plates. The normal development of A. punctatum has been standardized by a series of drawings made by Miss Krause, which, it is hoped, may be published later. The stages, referred to by number in the present paper, are represented by these drawings. They are briefly characterized in the text where necessary for clearness. A few are shown in outline (figs. 1, 2, 3, 4, 10, 22, and 23).

has grown to nearly the same length. By stage 38 it has become much longer and more club shaped, being clearly thicker at the end than near its attachment. Just before this stage is reached, the secretion of sticky mucus begins at the tip of the organ. From now on, the balancer simply elongates, its basal part becoming somewhat more slender (fig. 3).

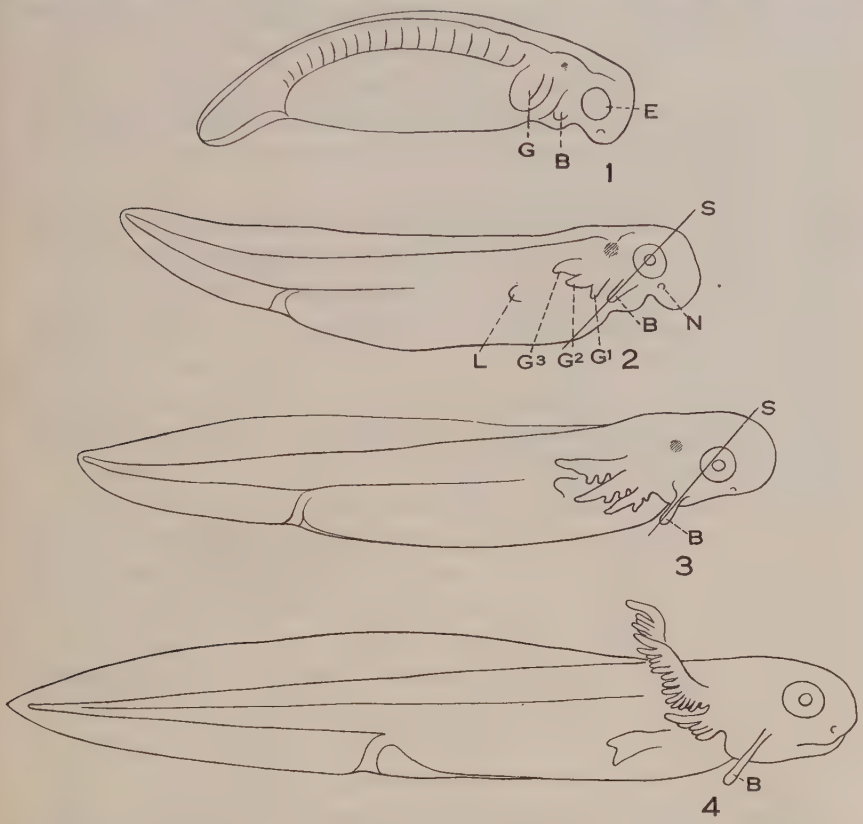
Examined by reflected light under the binocular microscope, minute glistening processes may be seen projecting from the epithelium covering the tip. When a needle is applied to this region, it will stick, and in this and the following stages it is also possible thus to turn the embryo into any position one desires by simply pulling on the balancer.[15] The stickiness of this secretion does not compare, however, with that of the mucus secreted by the cement organ of the frog embryo, and I have never seen an Amblystoma suspended in the water by the balancer as Rusconi figures the Triton embryo. The secretion seems to be most active between stages 38 and 43 (fig. 4). After the latter stage the rounded end is not so thick and the secretion of the mucus abates.

The balancer is broken off at its base at stage 46, when the animal begins to feed. Occasionally the larva has been observed to devour the cast-off appendage. The exact time at which it is shed varies slightly. Sometimes both balancers are gone when yolk is still plainly visible in the intestine, and at other times one is lost considerably before the other. When the larvae are crowded and frequently jostled by one another the balancers are more likely to be lost prematurely than when each larva is kept by itself in a dish. When one is missing the embryo is found always to lie partly on that side, as Egert and Latta have pointed out.

The balancer may be studied to great advantage if the embryo is anesthetized in chloretone and laid on its side under a water-immersion lens (preferably Zeiss D*), by which it can be conveniently examined at magnifications ranging from 350 to 450 diameters (fig. 48). The tip of the club-

[15] Cf. Egert, p. 284.

shaped organ is then found to be studded with spike-like projections, consisting of a hyaline substance, each from a single cell (*s.c*). These projections, which may be called



Figs. 1 to 4 Four normal stages of Amblystoma punctatum, showing the development of the balancer. *B*, balancer; *G*, gills; *L*, anterior limb; *N*, nasal pit; *S*, line of section in figs. 50 to 55, inclusive. × 8.

Fig. 1 Stage 34.

Fig. 2 Stage 37.

Fig. 3 Stage 39.

Fig. 4 Stage 43, showing the organ at the height of its development.

'secretion cones,' have previously been noted by Greil.[16] They vary considerably in their shape and length. Sometimes they are blunt rounded hillocks, sometimes cones slightly rounded at the end, and sometimes they are still

[16] Op. cit., p. 261.

more drawn out. They seem to show most characteristically about stage 43 (fig. 4), which was the age of the specimen shown in the figure. While nearly clear and translucent, they are nevertheless faintly granular, and at times pigment granules, found also in the ordinary epithelial cells, may be drawn out for short distances into the base of the hyaline cones. The cells which bear these processes are obviously the ones that secrete the mucus. Examined with the aid of the Barber microdissection apparatus, it is found that the whole surface of the tip of the balancer is covered by a very thick glairy mucus, which is sufficiently tenacious to bend the fine glass dissecting needles at least 90° and to hold them a short distance off from the surface of the balancer itself.

In these later stages the rest of the surface of the organ is covered by a more flattened epithelium, which has the same fine cuticular border (*cut*) as that covering the rest of the organism and is very smooth, reflecting the light very sharply. Occasionally a ciliated cell will be found.

The blood vessels (*b.v*), a single straight afferent vessel and a similar efferent one, may be readily seen. They are just wide enough for the passage of a single corpuscle at a time. They do not run quite to the tip, and often the two vessels are connected distally by a double loop. The diameter of the organ in stage 43 is approximately 0.14 mm., its length at the same stage being about 1.13 mm.

The rigidity of the organ is due in great measure to a curious skeletal structure located directly under the epithelium, the formation of which is described in the next section (p. 366). The nature of this structure has been differently interpreted by previous observers, Bell calling it a basement membrane, while Latta terms it unreservedly a dermal bone. It was, therefore, not without interest to study its composition by means of the various chemical tests applied by Mall ('96) to the study of the connective tissues. Specimens were prepared as follows: The larvae (stage 43–45) were anesthetized and the balancers removed. At the same time, for comparison, the lower jaws of the same individuals

were cut off and transferred with the balancers to glass slides. The teeth are distinct at this stage and are attached to the dermal bones of the jaw. These preparations were then treated with acids, caustic potash, and digestive ferments.

The membrane is clearly differentiated from bone by the action of caustic potash. A specimen mounted in water, viewed in optical section, shows it as a very distinct hyaline structure. If, now, a drop of 40 per cent caustic potash is drawn through under the cover-slip, for a few minutes the membrane remains clear, but soon the balancer shrinks and the membrane becomes invisible, with the epithelial cells dissociated. The dissolution of the membrane is complete in the strong alkali, and it is not restored on subsequent neutralization by acetic acid. The bones and teeth of the lower jaw, which are not very clear in water owing to the opacity of the underlying tissues, become very clear in caustic potash, and after a few hours may be almost completely isolated from the remains of the surrounding tissues which are disintegrated by the alkali. A balancer closely watched, as the caustic potash was drawn under the cover-slip, showed the epithelium to be dissociated very rapidly, leaving the basement cylinder bare and very clear. In two or three minutes, however, the latter began to swell irregularly along its outer contour, and almost immediately afterward it had entirely disappeared, leaving a core of much swollen and damaged mesenchyme.

The behavior toward strong acetic acid is quite different. When a specimen like the above is treated with glacial acetic acid, the balancer membrane stands out plainly as soon as the epithelium becomes partly dissociated and clear. The membrane, however, does not dissolve even when the action of the acid is continued for twenty-four hours. The teeth and membrane bones also stand out under this treatment more clearly than in the untreated specimen, but they are not so clear as in the potash, because the surrounding tissues are not destroyed to the same extent.

Dilute hydrochloric acid (0.5 per cent) has likewise no very marked action upon the balancer membrane nor upon the teeth and bones at this stage. The covering epithelium and the surrounding tissues are not very much changed by this reagent even after treatment lasting days.

When subjected to digestion with pepsin combined with 0.5 per cent hydrochloric acid, the epithelial cells swell quite rapidly, but at first there is not much dissociation. The balancer membrane, however, becomes indistinct in twenty or twenty-five minutes, and in the course of an hour fades completely. The same is also true of the teeth and bones. All of these structures seem to be quickly and totally dissolved by this treatment.

Pancreatin dissolved in 0.3 per cent solution of sodium carbonate acts very differently from the pepsin. The epithelial cells are dissociated in the course of half an hour. The cells become swollen and clear, but the balancer membrane remains intact; likewise the teeth and bones become very distinct. In the course of two hours the cells are disintegrated, and on the day following the beginning of treatment there is practically nothing left but the membrane bones with the teeth attached and the balancer membrane. The latter may be isolated whole throughout its entire length. It is like a long narrow test-tube, glass clear, very thin at its peripheral end where the mucus of the balancer is secreted, and flaring in funnel fashion at its basal attachment (fig. 49).

When one surveys these reactions, which were repeated a number of times and found to give uniform results, one finds that the balancer membrane is clearly differentiated from bone by its behavior toward strong caustic potash, just as it is histologically differentiated from bone by the absence of a distinct layer of osteoblast cells. In its behavior toward the digestive ferments and toward acetic acid it is similar to that of the membrane bone of the jaw and the dentine of the teeth. The difference between the membrane and bone, as regards reactions to alkali, is not due merely to the possible presence of mineral salts in the bone, because it has been found that

bones which have been treated with caustic potash and subsequently treated with acetic acid still remain intact, though they become flexible and somewhat warped.

The reactions of the balancer membrane to the digestive ferments correspond to those of white fibrous tissue rather than to those of elastic tissue, but, on the other hand, the reaction with acetic acid is like that of elastic tissue and differs from that of white fibrous tissue. The membrane, in fact, behaves more like reticulum, as described by Mall, than like either elastic or white fibrous tissue, but it is less resistant to peptic digestion than reticulum. This is possibly due to its embryonic character.

Normal development as seen in sections

The changes which initiate the development of the balancer first become visible about stage 33, at which time the inner layer of the ectoderm covering the mandibular region begins to thicken. By stage 34 (fig. 1) this process has proceeded so far as to cause a rounded bulging on the surface. The cells are now distinctly columnar with oval nuclei, forming a single row near the outer surface and leaving a considerable layer of cytoplasm, laden with fine yolk granules, within. The covering layer (Deckschicht) of ectoderm is also thickened, but far less markedly than the inner layer. The inner surface of the ectoderm is scarcely evaginated, and there are no local changes visible in the underlying tissues. By this time the process from the ganglion crest has grown down and wrapped itself completely around the mesoderm of the mandibular arch,[17] and it is this mesectoderm that lies directly under the thickened epithelium and is destined to supply the mesenchymal core of the balancer.

Slightly later (fig. 50, *e.s* and *e.d*) this modified ectoderm projects farther from the surface of the head, and the underlying mesectoderm (*m.e*) fills the space formed by the evagination. The hyomandibular artery (*a.h*) of Maurer (first aortic

[17] Cf. Landacre ('21) and Stone ('21, '22).

arch) is now well developed and may be seen skirting the base of the balancer bud. In sections stained in Mallory[18] the beginning of the basement membrane (*b.m*) shows as a very distinct blue line between the inner border of the epithelium and the mesenchyme. It is only in the balancer that this membrane is seen at this stage. The rest of the ectoderm rests directly upon the mesoderm with nothing more definite than an indistinct cell membrane intervening. From this stage on it has proved a great advantage to cut the sections in a plane running at an angle of about 45° to the transverse, thereby cutting the balancer longitudinally.

During this period the principal changes taking place are the rapid lengthening of the balancer and the condensation of the basement membrane (fig. 51), which becomes thicker except at the tip. In places where it has shrunken away from the epithelium its adherence to the mesenchyme shows plainly. Where it is cut tangentially, it shows as a fine reticulum stained intensely blue. The inner layer of the covering ectoderm is still columnar, but its thickness is already markedly diminished in proportion as the appendage has increased in length.

Sections through an embryo in stage 37 (fig. 2) show the relations of the constituents of the balancer in diagrammatic clearness.[19] The epithelium is very regular, still consisting of an inner columnar and an outer cuboidal layer, the latter being about two-thirds or three-quarters as thick as the former. The basement membrane is considerably thicker than before, becoming very thin, however, at the tip. At the base the membrane flares out, funnel-like, curving with the contour of the epithelium around the base of the appendage. Here it is frayed out into very thin fibrillae running out into the mesenchyme. The limiting membrane of the ectoderm (membrana terminans of Merkel) is beginning to show elsewhere in the embryo, particularly in the anterior portion of the head. The core of the balancer contains scattered cells, but

[18] A. punctatum, series 59 (stage 35) and series 51 (stage 36—).

[19] A. punctatum, series 57 (stage 37).

at the distal end there is a more dense aggregation. The blood vessels take up a good deal of space in the organ. A fine nerve, derived from the Gasserian ganglion, may be traced almost to the tip.

By stage 38 the balancer has more than doubled its length, compared with that of the preceding stage, the increase being from 0.2 to 0.43 mm., as measured along the medial border in the sections. At the same time its diameter at the base has decreased from 0.13 to 0.09 mm., but at the tip it remains unchanged, the organ having become club shaped (fig. 52 and 52A). The decrease in diameter is due entirely to the stretching of the ectoderm, which is but half as thick as previously, while the diameter of the core including the basement membrane remains unchanged. Corresponding to this diminution in thickness, the cells of the basal layer have changed from columnar to cuboidal in shape, those of the covering layer from cuboidal to flattened. At the outer end, however, the cells still retain their former respective shapes. The mesenchyme has scattered considerably, but it still remains more dense at the tip than proximally. The nerve (*n*) is very distinct. However, its exact mode of ending cannot be made out, and no special sensory cells have been found in the epithelium. The basement membrane (*b.m*) has become thicker. The connection of the efferent vein with the jugular can now be made out as originally described by Maurer ('88). It probably existed earlier.

An unusually fine specimen,[20] very slightly older, shows a little further differentiation of tissues, but the length of the balancer is the same. The cuticular border of the ectoderm is sharply defined and is colored more intensely blue in Mallory's stain than previously. This is particularly true in the cells at the tip of the balancer. By this time the secretion of mucus has begun in the live embryos, and the change in the staining properties of the border is no doubt correlated with this function (p. 360). The basement membrane shows with extraordinary clearness. It is thickest near the

[20] A. punctatum, series 62 (stage 39—).

base and tapers gradually toward the tip where it is no thicker than the basement membrane generally in other parts of the head. The funnel-like expansion of the membrane is becoming separated from the epithelium and shows clearly as belonging to the mesenchyme (fig. 53). It is frayed out into fine fibrils which, like the general basement membrane and the thickened part of the membrane in the balancer, stain much more intensely blue than the general mesenchymal network. One thing which should be emphasized is that the mesenchyme cells have no special relation to the balancer membrane. Here and there such a cell may lie close to the membrane, but there are long stretches quite devoid of cells, as also in earlier stages.

There is a considerable advance in stage 40[21] over the condition found in the last specimen. This is most noticeable in the differentiation of the cartilages of the head, which are beginning to show their characteristic matrix, particularly in the palatoquadrate and parachordals, where the matrix appears as a dense reticulum stained intensely blue. The palatoquadrate is close to the base of the balancer, with which it is connected by a less dense mesenchyme, and the nerve to the balancer runs through a foramen in this cartilage. The proximal part of the balancer membrane (fig. 54, *b.m*) is more detached from the epidermis than before. It lies embedded in the mesenchyme, and there are even a few cells between it and the epithelial layer. Throughout the free portion of the balancer the membrane remains in contact with the ectoderm. It is slightly thicker than before, though still remaining thin at the tip. There is now a very distinct membrane, the beginning of the corium underlying the ectoderm all over the head, except possibly on the gills. The epithelium of the balancer is now considerably more flattened, especially near the base. The growth in length of the organ is apparently met almost entirely by a change in shape of the epithelial cells rather than by cell multiplication. Mitoses, though occasionally found, are rare. The outer border of the

[21]A. punctatum, series 64.

epithelium is again stained intensely blue, and this is especially marked near the tip, where a few of the blunt cone-shaped processes ('secretion cones'), seen in the fresh specimens, (fig. 48) are in evidence. They constitute part of the border and are themselves heavily stained.

An embryo in stage 41[22] shows very clearly a condensation of the mesenchyme running from the palatoquadrate cartilage (fig. 55 *p.q*) over to the rim of the balancer funnel, the expanded basal end of the membrane. This is an extension of the chondrification, but the matrix is not so highly differentiated, being more reticular than that of the cartilage proper. It extends to the rim of the funnel, uniting the latter firmly to the palatoquadrate. This process forms a cup-shaped cartilaginous support or articular process (*a.p*) for the balancer. There are two notches in the rim of the cup, one anterodorsally for the vein and the other ventroposteriorly for the artery. In this specimen the nerve runs through the opening alongside the vein, whereas in the previous one it passed through a separate foramen. The upper opening is bridged over by cartilage, the rim of the balancer funnel not being exposed; i.e., there is a true foramen through the cartilage. The lower opening lies between the quadrate and the rim of the funnel, the latter not having any direct cartilaginous support at that point.

Compared with that of the previous stage, the balancer is somewhat longer and a little thinner in its proximal part, where the epithelium is still more flattened than before. At the tip the inner layer is still columnar, and the secretion cones of the outer layer are very distinct. Oddly enough, these cells stain only very lightly with Mayer's mucicarmine; in fact, the tissue having the greatest affinity for this stain is the cartilage matrix. However, the cells of the outer layer of the cement organ of Rana sylvatica have still less affinity for this stain, remaining almost colorless after treatment with it, although the same stain tested with submaxillary gland gives the characteristic reaction.

[22]A. punctatum, series 66.

From this time until the balancer begins to show signs of degeneration in stage 45 there is not much further change. By the time the latter stage has been reached, the articular cup has become fully differentiated into cartilage (fig. 56, *a.p*) and there is then a thin layer of this tissue bridging over the posteroventral notch, so that the base of the balancer membrane is firmly attached to cartilage around its entire circumference.

In this stage preparations for the casting off of the appendage may be detected.[23] In one case on the left side the balancer membrane is constricted where it passes through the epidermis, as if it were about to be pinched off. On the opposite side this condition does not show. In another case there is only a slight dent in the membrane on both sides. In a third specimen the membrane is broken anteriorly with an irruption of epithelial cells into the cleft. The general appearance of the balancer in the live animal at this stage is somewhat ragged. At the tip the cells of the ectoderm are no longer so regular, and some of them show degenerative changes, being filled with coarse granulations. Secretion cones show very plainly, but are not so numerous.

Between stages 45 and 46 the balancers are shed. One specimen[24] with one balancer still attached (fig. 56) shows the membrane partly broken through by an irruption of epithelium (*e.p*), by which the nerve (*n*) and blood vessels (*b.v*) are cut off. The cup-shaped articular process (*a.p*) of the palatoquadrate is at the height of its development, no regressive changes yet being noticeable. Distally the epithelium contains numerous degenerating cells with granulations. On the right side the balancer is gone, but a solid papilla of ectoderm is left projecting from the surface. Another case[25] also has but one balancer. In place of the one cast off there remains likewise an epidermal papilla, together with a plug of epithelial cells extending into the funnel. The nerve may be

[23] A. punctatum, series 72, 81, and 77 (stage 45).
[24] A. punctatum, series 76.
[25] A. punctatum, series 82.

followed into this plug for some distance. The remaining balancer is attached only by the epithelium, which has completely cut through the membrane and the blood vessels and nerve inclosed within it. A third specimen[26] shows both appendages gone, each having been severed evenly with the surface of the head (fig. 57). The funnel-like portion of the membrane (*b.f*) is left embedded in the mesenchyme, as in the other cases. Reaching into the mouth of the funnel, there is a distinct epithelial plug (*e.p*) to which the balancer vessels (*b.v*) and nerve (*n*) extend. These cases show clearly that the casting off of the balancer is a process of autotomy, prepared in a regular way by the action of the tissues of the organism, and is not due merely to an accidental blow from without.

The basal portion of the membrane, which remains after the balancer is cast off, persists for a long time. A number of specimens, preserved from one to three weeks after the shedding of the appendage, all show the presence of this funnel (fig. 58, *b.f*), through which a nerve (*n*) and a small blood vessel run. The former is distributed to a considerable area of the skin overlying the articulation of Meckel's cartilage with the quadrate, and is obviously the balancer nerve which has regenerated after its natural amputation and now supplies a different area from before. The basal funnel itself lies simply embedded in the connective tissue, and the mesenchyme cells show no definite relation to it, the articular cup having atrophied. The oldest specimens examined[27] were preserved about sixty-five days after the yolk was resorbed and the balancer shed. They also showed the presence of the membrane and essentially the same relation of the surrounding parts.

In all of the above cases the cartilaginous articular process of the palatoquadrate, which had attained its maximum degree of development about the time the balancer was shed, has become dedifferentiated. The funnel has at best only a

[26] A. punctatum, series 75.

[27] Exp. Tr.E.61+ and Tr.E.61—.

fibrous connection with the cartilage and for the most part lies surrounded simply by diffuse mesenchyme. The dedifferentiation of the articular cup takes place quickly after the balancer drops off. In a case[28] preserved about eight or ten days after this event the cup has already been reduced to a fibrous condition. The cartilage matrix has disappeared, and the cells are flattened as in the perichondrium or in ligaments. This seems to be a true case of regressive development, and a reversal of the histogenetic process. There is no evidence of degenerative changes in the cartilage of the cup. It simply loses the specialized character of cartilage, being reduced first to a dense fibrous condition and then to a more diffuse mesenchyme.

EXPERIMENTAL

A general survey of the normal development of the balancer, as described above, shows that the first visible changes involve the ectoderm which thickens markedly just behind and below the eye, owing chiefly to the change in shape of the cells of the inner layer from cuboidal to columnar. This is followed by the formation of an evagination into which cells push out from the underlying mesectoderm, which in turn is derived from the neural crest. The organ elongates, and this, too, is brought about by a change in shape of the epithelial cells rather than by any marked cell proliferation. While the evagination is taking place, a very distinct basement membrane, the most striking feature of the organ, begins to form in the mesenchymal ground-substance immediately underlying the modified ectoderm. The membrane is clearly formed by a condensation of the intercellular ground-substance, the mesenchyme cells showing no definite relation to it. After a time the basal portion of this membranous tube becomes separated from the ectoderm and acquires an attachment to a cartilaginous cup-shaped articular process, which is developed on the palatoquadrate. A branch of the hyomandibular artery enters the balancer at an early period and the

[28] Exp. E.E.28.

return vessel establishes a connection with the jugular vein. A nerve from the mandibular division of the trigeminus is also a constant feature of the appendage.

The main question now before us is how these different factors are correlated in development. Bell's original experiment showed that the balancer is determined primarily in the ectoderm, and the present study has confirmed and extended this result. Some of the specific questions remaining to be answered are: When does this differentiation take place? To what extent are this area of ectoderm and the underlying tissues specialized? What is the nature of the action originating in the ectoderm, and what the nature of the response called forth in the mesenchyme which gives rise to the basement membrane? Is the formation of the articular process on the palatoquadrate cartilage dependent upon the presence of a balancer, or is it an independent property of the palatoquadrate itself? Is the attraction between the balancer and the nerve a specific one? To what extent is the interaction between the ectoderm and the mesenchyme limited as to time? Can the ectoderm alone of A. punctatum call forth the development of a balancer in species which normally have none?

To answer these questions experiments have been made with the following results:

1. Transplantation of balancer ectoderm to other regions of the embryo in a series of embryos ranging from those with medullary plate to those just prior to the external appearance of the balancer (stages 15–33); with certain limitations (p. 382) this was followed by the development of the balancer in the abnormal location.

2. Removal of the ectoderm of the balancer region and replacement with ectoderm taken from some other part, resulting in suppression of development.

3. Removal of the ectoderm of the balancer without replacement, frequently, but not always, followed by regeneration.

4. Amputation of the balancer which has already developed, no regeneration taking place.

5. Removal of the mesoderm of the mandibular arch, without effect upon the development of the balancer. The same result follows the removal of the ganglion crest.

6. Change of orientation of the balancer ectoderm, followed by abnormal direction of outgrowth, which, however, does not seem to be correlated exactly with the orientation of the graft.

7. Transplantations between embryos of different ages—old ectoderm to younger embryos and young ectoderm to older embryos—with the result that the time of development is found to be determined by the age of the graft.

8. Heteroplastic grafts: reciprocal grafts of balancer ectoderm between Amblystoma punctatum and A. tigrinum, resulting in the development of a normal balancer from punctatum skin implanted upon tigrinum embryos and suppression of development in the reciprocal combination; similar grafts of ectoderm from A. punctatum placed in the head region of embryos of R. sylvatica, resulting in imperfect development of the balancer.

Development of the balancer in abnormal location

In Bell's experiments ectoderm from the proper region, transplanted to various locations on the head or trunk of the embryo, just before the balancer would have normally appeared, gave rise to this organ in its new surroundings. The present work shows that the balancer ectoderm is differentiated very much earlier. Several experiments, made in 1919 and directed toward the study of the gills, proved that ectoderm from the front part of the head, taken from an embryo with wide open medullary folds (stage 15–16), would give rise to a balancer in the new position. However, there are apparently secondary factors influencing the formation of the balancer, such as the location of the graft and the stage at which it is removed from its original seat, as will be seen when the variants of this fundamental experiment are considered.

Development of the balancer on the side of the head, especially in the gill region. The cases under this heading may be divided into two groups according to the age of the embryos from which the grafts were taken. Those in which the donor embryos were still with open medullary folds will be considered first.

Embryos of this early period are very sensitive to operations and the mortality is very high. For this reason the grafts alone were taken from young embryos, which varied

TABLE 1

Result of transplanting ectoderm from the anterior part of the head, including the covering of the mandibular arch, to the gill region

A. Grafts taken from embryos with open medullary folds

	NUMBER OF EXPERIMENTS	VALID EXPERIMENTS	BALANCER APPROXIMATELY NORMAL	BALANCER DISTINCTLY ABNORMAL	BALANCER ABSENT
Graft free from mesoderm	5	5	4	1	0
Graft not quite free from mesoderm	11	10	8	2	0
Presence of mesoderm on graft undetermined	6	4	2	1	1
Total	22	19	14	4	1

B. Grafts taken from embryos with closed medullary folds

Graft free from mesoderm	16	8	4	4	0
Graft not quite free from mesoderm	2	1	1	0	0
Presence of mesoderm on graft undetermined	2	1	1	0	0
Total	20	10	6	4	0

from just below stage 15 (medullary plate) to stage 19 (medullary folds approximating), while the hosts varied from stage 20 (folds not yet quite closed in head region) to stage 24. Most of them were in stage 21 or 22, i.e., with folds just closed. In some of the cases care was taken to remove no mesoderm with the ectodermal flap, and in others scattered mesoderm cells stuck to the epithelium. No essential difference was found between the two groups of experiments.

The results have been summarized in table 1 A, from which it is seen that a normal or nearly normal balancer developed in

the territory of the grafted ectoderm in fourteen cases out of nineteen and that it failed entirely to develop in only one case.[29] In two experiments it was much involved with the gills[30] and in two others[31] a blunt balancer process was formed, but the appendage never fully developed. One of these, examined in sections, showed the characteristic epithelium and basement membrane spread out and not extended to form a long process. The grafts in five of the cases certainly had no mesoderm sticking to the ectoderm when transplanted. Four of these are among the fourteen having well developed balancers.

Histories of several cases follow:

Experiment CEBr.10. The head ectoderm from an embryo in stage 15 (medullary plate) was transplanted to the gill region of an embryo of stage 23. The anterior border of the graft, which was originally on the anterior midline of the embryo, became the ventral border after transplantation. Six days after the operation the grafted balancer was first noticed, and three days later it had become a long process (fig. 8). The embryo was preserved thirteen days after operation at about stage 43 and subsequently cut into transverse sections. The epithelium of the grafted balancer is typical, and underlying it there is a thick basement membrane. The basal part of the latter is not much expanded nor much separated from the epidermis, and there is no cartilage near enough to serve as its attachment. Blood vessels are present, but are much congested, causing a distention of the base. A distinct nerve can be followed well into the organ. It arises from the r. jugularis of the facial nerve and is probably from fibers reaching this trunk from the ninth through the connecting commissure.[32]

Experiment CEBr.14. Operation as in previous case except for slight differences in the ages of the embryos: donor stage 16 (low medullary folds), host stage 20 (folds not quite closed in head). Four days after operation (fig. 5) the extent of the graft was seen to include the branchial region and the posterior slope of the mandibular arch. Three sprouts grew out in the gill region, but only the last one developed into a fully formed gill. The second was reduced in size, and the first became a balancer (fig. 6, *b'*). Even the normal

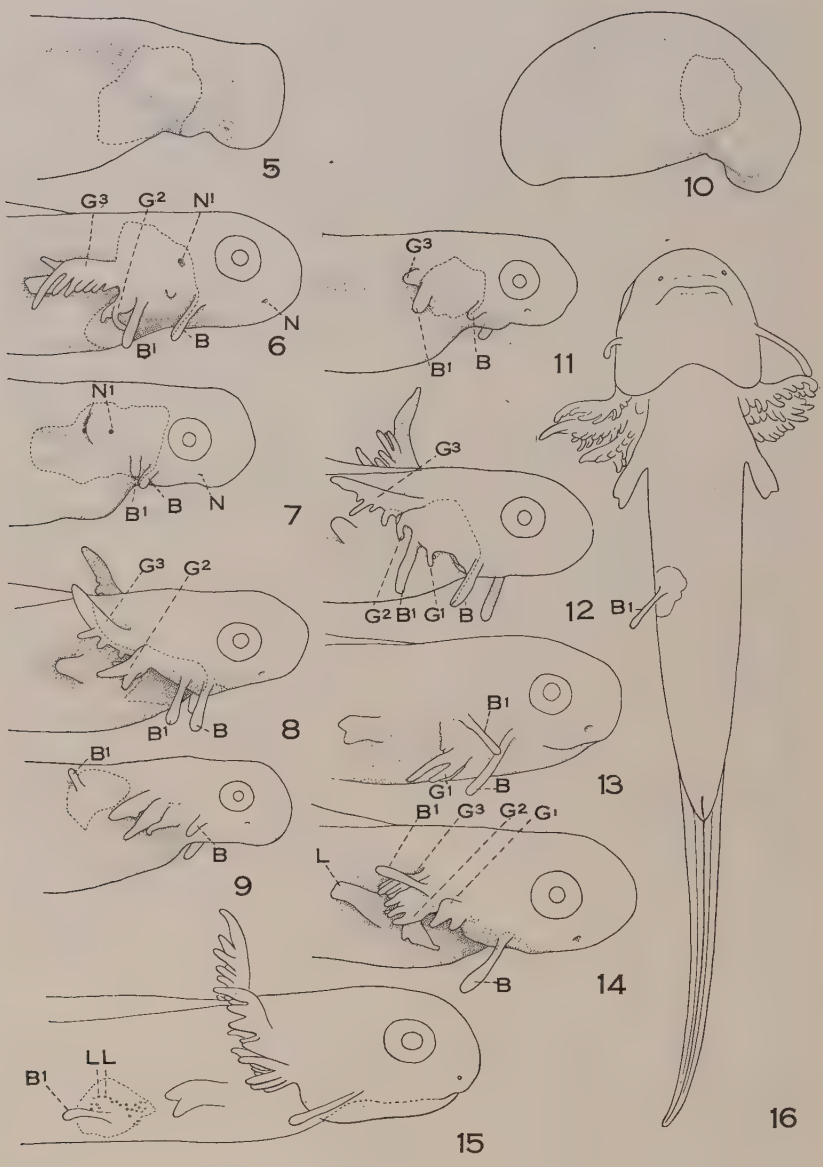
[29] Exp. CEBr.9.
[30] Exp. CEBr.8 and CEBr.20.
[31] Exp. CEBr.18 and CEBr.28.
[32] Cf. Coghill ('02).

balancer was partly covered by the graft. The specimen was preserved thirteen days after the operation. In sections the base of the grafted balancer is wide; it arises, in fact, from a distinct projection, probably the representative of the first gill. The basement membrane is distinct, and the rim of the funnel is separated from the ectoderm, as it is normally. Vessels do not show distinctly in the sections, but a note made from the living specimen the day before preserving shows that a vessel with stagnant blood was then present in the balancer. There is probably no nerve.

Experiment CEBr.18. Operation as before except that the orientation of the graft was uncertain; donor embryo stage 19 (medullary folds approximating); host stage 23; ectoderm free from mesoderm cells. The specimen was preserved six days after operation, stage 38. At this time the third gill was normally developed, but in place of the other two there was merely a large and somewhat irregular nodule. Sections through the latter indicate that it represents the balancer, which has failed to elongate and has remained spread out (cf. fig. 64, drawn from a similar specimen). It has the characteristic epithelium, underlying which is the basement membrane. An endodermal process (visceral pouch) extends out toward the nodule.

Experiment CEBr.19. Donor embryo stage 19, host stage 23; oval graft normally oriented; some mesoderm taken with the ectoderm. The surface of the transplanted skin was uneven and the gill mass developed irregularly. Three gills are present (fig. 14). The first and third are small, and the second bends anteriorly. Growing out from the dorsal surface of this gill near its base is a large well-developed balancer. The specimen was preserved eleven days after the operation in stage 41 and cut into transverse sections. The balancer epithelium is normal, and the basement membrane is present, but the basal part of the latter is much spread. A nerve, which may be traced to the vagus, and blood vessels enter the appendage. This case is remarkable for the high degree of development of the gills, especially anteriorly, and for the close relation between balancer and gills.

The above cases are fairly representative of the group. They show that while the balancer may grow in the gill region almost normally, in many cases it is somewhat affected by its strange environment, not lengthening to its fullest extent and having its base enlarged. In these cases the balancer is superposed upon the gills. The epithelium maintains its specific character and brings about the formation of a typical basement membrane in the underlying mesenchymal matrix;



Figs. 5 to 16 Cases of supernumerary balancers developed from transplanted ectoderm. The dotted line shows the boundary of the grafted tissue. *B*, normal balancer; *B'*, balancer developed from grafted ectoderm; *N*, normal nasal pit; *N'*, grafted nasal pit; G_1, G_2, G_3, first, second and third gills, respectively; *LL*, lateral-line sense organs; *L*, fore limb. × 10.

at the same time the epithelium immediately surrounding it, which is closer to it than the gill rudiments are normally, is able to produce gills, though not in perfectly normal form. Processes of differentiation which normally are localized according to a definite pattern and do not interfere with one another are thus brought to some extent into conflict.

The cases in which the grafts were taken from embryos with closed medullary folds will now be considered. These experiments, which belong to several different series, vary more in the mode of procedure than the previous group. The

Fig. 5 Exp. CEBr.14, four days after operation. The ectoderm was taken from the anterior part of an embryo in stage 16 (low medullary folds).

Fig. 6 Same case, twelve days after operation.

Fig. 7 Exp. TrAk.3, in which the transplanted ectoderm was taken, at stage 20 (medullary folds not quite closed) from across the midline of the head, including both nasal pits and the covering of the mandibular arch. The figure, drawn five days after operation, shows one supernumerary balancer close to the normal and the two nasal pits. The other supernumerary balancer did not develop.

Fig. 8 Case similar to one shown in figures 5 and 6, nine days after operation. Exp. CEBr.10. Grafted ectoderm in this case taken from an embryo at stage 15 (medullary plate).

Fig. 9 Embryo six days after operation with balancer just above pronephros, developed from ectoderm grafted in stage 26. Exp. BPN.4.

Fig. 10 Embryo in stage 26, sketched twenty hours after operation, showing boundary of ectoderm (broken line) grafted from anterior region of head. Exp. ABr.2.

Fig. 11 Same embryo four days later, showing supernumerary balancer bud and the beginning of the third gill.

Fig. 12 Same individual five days later (ten days after operation). Supernumerary balancer is involved with the first and second gills.

Fig. 13 Individual with supernumerary balancer in the gill region developed from ectoderm transplanted at stage 21 (medullary folds just closed). Exp. CAVBr.1. Drawn from specimen preserved fourteen days after operation.

Fig. 14 Larva with supernumerary balancer growing from base of second gill developed out of ectoderm transplanted at stage 19 (medullary folds approximating). Exp. CEBr.19, eleven days after operation.

Fig. 15 Balancer developed on the flank from ectoderm grafted at stage 32 (shortly before the normal appearance of the balancer). Exp. BFl.8. Figure drawn eleven days after operation. Numerous lateral-line sense organs have developed from the grafted ectoderm.

Fig. 16 Individual with supernumerary balancer on abdomen developed from a graft which included both ectoderm and mesectoderm, taken at stage 26 + (fig. 10). Exp. BFl.17. From specimen preserved ten days after operation.

results as regards the external development of the balancer are summarized in table 1 B.

One case,[33] in which the graft was placed more dorsally than usual, shows the balancer in a correspondingly higher position (fig. 13) and pointing anteriorly, which is unusual. Blood vessels, but no nerves, were found.

Five other cases had an oval area of ectoderm from the posterior part of the eye, the mandibular arch and part of the branchial region placed farther back, so as to cover the entire branchial swelling. In only two of these[34] did a balancer develop from the graft (figs. 10, 11, and 12, showing three stages of the same specimen). In three others what purported to be a balancer bud appeared in due course, but it grew no further. None of this series have been examined in sections.

In several experiments the ectoderm covering the whole front of the head on both sides was transplanted to the right side over the gill region and posterior to it. The graft thus included both balancer rudiments, one of which came to lie just behind the balancer of the host, and the other much farther back, even beyond the gill region. No mesoderm was left sticking to the graft, but in most cases a few neural-crest cells were left along the midline. There was a high mortality in this series and only four cases lived. One balancer, the anterior one, developed in three of these (fig. 7), but no trace of the posterior one could be found in the two specimens that were examined in sections. The supernumerary balancer was partly fused with the normal one of the host in one of these cases. In the fourth specimen blunt processes, supposedly balancers, were formed in stage 37–39 at the time when the normal balancer was growing rapidly, but they did not elongate. The gills were entirely lacking in all of these cases.

Sixteen of the above cases with normal or approximately normal balancers together with three others described in the

[33] Exp. CAVBr.1.

[34] Exp. ABr.2 and ABr.5.

next section have been examined with regard to innervation. Nerves were found in thirteen of these. They were traced to the facial in two,[35] to the glossopharyngeal in one,[36] and to the vagus in five.[37] In each of two other cases[38] a fine nerve was found entering the balancer, but its central connection could not be traced with absolute certainty; in all probability it was from the vagus. In one case[39] with the balancer grafted in the region of the pronephros, the innervation was from a spinal nerve. In another,[40] where the balancer grew on the side of the body from a patch of skin which undoubtedly included a placode, the innervation (fig. 62) was from a small nest of ganglion cells, in all probability derived from the graft. Still another individual[41] with the balancer on the flank showed a nerve, probably of spinal origin (fig. 63). These results show that the innervation of the balancer is not necessarily specific. The organ acquires its innervation from the nerve running nearest to its base, which, so far as has been determined, may be a spinal nerve or any one of three cranial nerves. Even in two cases in which Amblystoma ectoderm was grafted to frog embryo nerves were found running into the balancers that developed.

In thirteen of the sixteen cases blood vessels were found in the grafted balancer, but there are no observations to indicate whether there was any circulation of the blood.

Development of balancer in the pronephric region and on the flank. A patch of ectoderm, including the balancer rudiment, was transplanted to the pronephric region in four cases, the donor embryos being in stage 25 or 26 and the recipients a little older. The ectoderm was free from underlying cells in all cases. The balancer developed in only one individual[42]

[35] Exp. CEBr.10 and Tr.Ak.3.
[36] Exp. CEBr.16.
[37] Exp. CEBr.15, 19, 21, 26, and 27.
[38] Exp. CEBr.24 and 29.
[39] Exp. BPN.4.
[40] Exp. BFl.8.
[41] Exp. BFl.17.
[42] Exp. BPN.4.

out of the four. This specimen (fig. 9) was preserved at stage 41 thirteen days after the operation. The balancer was somewhat more slender and shorter than the normal, and no circulating blood was ever seen, nor did sections reveal any blood vessels entering it. A nerve fiber from one of the spinal nerves may be traced for some distance from the base. The membrane is well-developed, but the basal funnel is not separated from the ectoderm. The balancer epithelium is normal, although the tip is smooth and without secretion cones. This is the only case among the transplantations in such young stages, where the balancer developed from a graft placed outside the head region.

The transplantations of balancer ectoderm to the flank were made in three different groups with a view to determining the effect of the underlying mesectoderm and of the age of the ectoderm on the results. Twenty-seven experiments were made, all of which lived and developed satisfactorily.

The first series of twelve experiments consists of those in which the ectoderm devoid of all underlying tissue was transplanted in stages varying from 24 to 28. In eleven of these the graft remained flat, no sign of a balancer developing. In one case only[43] a blunt cone was formed from the grafted ectoderm. Sections show that the ectoderm, instead of evaginating, has formed a solid club-shaped ingrowth and that there are traces of a balancer membrane. This case must be regarded as showing an abortive attempt to form the balancer.

When the underlying mesectoderm is grafted along with the ectoderm in the same stages as above, the results are different. Seven experiments of the kind were made, resulting in well-developed balancers in four cases and in abortive ones in three. In the latter the grafted ectoderm is thickened and bulges slightly and the basement membrane is somewhat thickened; besides, there are irregular bits of denser reticulum in places, remnants of the normal balancer membrane. The mesenchyme underlying the graft is denser than elsewhere.

[43] Exp. BFl.16.

In one of these cases[44] there is a distinct balancer bud, lined by a thick membrane, sticking out from the middle of the graft. Underlying it is a nodule of procartilage developed from the grafted mesectoderm. One of the cases[45] in which balancers developed (fig. 16) was cut into sections. The membrane is well formed, but the basal funnel is small (fig. 63). A nodule of cartilage (*c*) developed from the transplanted mesectoderm lies close to its attachment. A small nerve (*n*) is present. Its origin could not be traced, but it is probably a branch of one of the abdominal nerves, since no ganglion cells are present near the graft.

In eight cases ectoderm, free from underlying cells, was transplanted in embryos of stage 32–33. Seven of these gave rise to well-developed balancers. The remaining case showed a distinct balancer bud early in its history, but this remained as a small nodule. Sections of this specimen show a rudimentary balancer membrane, considerably thicker than elsewhere, and a small condensed mass of mesenchyme lying beneath.

Several of the last group of cases in which balancers developed were likewise examined in sections. In one of these individuals[46] operated in stage 34 and preserved five days later at stage 39, a cyst formed under the graft, due probably to occlusion of the pronephric duct. This was punctured and a permanent fistula developed. The balancer in this case was not normal, its surface being wrinkled. Sections show that it is not filled with mesenchyme, but contains two larger clear cavities almost devoid of cells. There is no complete membrane, but the cavities are lined in places by a thin membrane which extends as an irregular dense reticulum through the epithelium at the base of the balancer. This is in many respects like the cases in which the balancer develops in the stomodeal region (p. 385).

[44] Exp. BFl.21.
[45] Exp. BFl.17.
[46] Exp. BFl.6.

In another specimen,[47] preserved at stage 43 (fig. 15) eleven days after the operation, the balancer was slightly bent at the tip and somewhat enlarged at the base. In the graft a large number of lateral-line sense organs (*l.l*) had developed, which showed in life the intense blue color of the stained tissue. Evidently a placode had been transplanted with the ectoderm. The sections (fig. 62) show a balancer well filled with normal-looking mesenchyme with a distinct membrane (*b.m*) extending to the tip, where it is quite thin as it is normally. The epithelium is regular and at the end there are one or two small 'secretion cones.' Three days prior to preservation, when the embryo was in stage 40,

TABLE 2

Summarizing the results of experiments in which the balancer ectoderm was transplanted to regions other than the head

TYPE OF EXPERIMENT	RESULT		
	Balancer well-developed	Development abortive	No balancer
Ectoderm alone from embryos stage 24–28.....	1	1	14
Ectoderm and mesectoderm from embryos stage 24–28	4	3	0
Ectdoerm alone from embryos stage 32–33.....	7	1	0

numerous 'secretion cones' were visible in the living specimen. A nerve (*n*) enters the balancer, arising from a group of ganglion cells which lie embedded beneath the abdominal muscles and which must have taken origin from the placode included in the graft. No distinct blood vessel can be traced into the balancer, but there are spaces here and there within it that look like vessels.

Viewing the experiments of this section as a whole (table 2), we find that when the mandibular ectoderm is taken from embryos in stage 28 or younger and transplanted to a region other than the head, the balancer does not develop. There is only one outright exception to this rule out of sixteen cases, one other case showing an abortive development. This

[47] Exp. BFl.8.

is very different from the result obtained after transplantation to other parts of the head, even when the graft is taken from much younger embryos. The main difference between the substrate of the graft in the two series of experiments is the presence of mesectoderm on the side of the head and its absence from the flank. This seems to indicate that there is some reaction of a general nature between the ectoderm and the underlying mesectoderm necessary to produce a balancer—an hypothesis borne out by the second group of experiments described in this section. Here the mesectodermal substrate was transplanted with the ectoderm, with the result that in four cases out of seven balancers developed, while in the other three development began, but was arrested.

If, however, the differentiation has gone further, even though the balancer is not yet visible, then the ectoderm when transplanted alone is able to evaginate and to bring about the formation of the basement membrane from the ordinary mesenchymal matrix of the trunk region, as well as from the mesectodermal tissue.

In other words, the ectoderm of the balancer is specific and localized, but it only becomes effective in producing this appendage after it has reacted for a time with the mesectoderm. For the actual formation of the basement membrane, ground-substance of either type of mesenchymal tissue is sufficient.

The balancers which develop in these cases are usually shorter than the normal, but sometimes they are of nearly normal length and thickness, and this in spite of uniformly negative observations with regard to blood circulating in them.

Development of the balancer in the stomodeal region. The purpose of this group of experiments was to exclude mesenchyme, either mesodermal or mesectodermal, from access to the developing balancer, in order to determine the effect of the lack of this tissue upon the evagination of the ectoderm and upon the formation of the basement membrane. The high mortality in the group reduced the number of available

cases from eighteen to nine, but the results of the latter are interesting, in spite of the fact that complete exclusion of mesenchyme was not attained. Aside from its effect upon the balancer, the operation brings about far-reaching abnormalities in the mouth region, as described more fully by Miss Adams ('24).

The nine cases fall into three classes: those in which fully formed balancers developed—three cases; those in which the balancer was collapsed or shrunken—four cases, and those in which no development occurred—two cases.

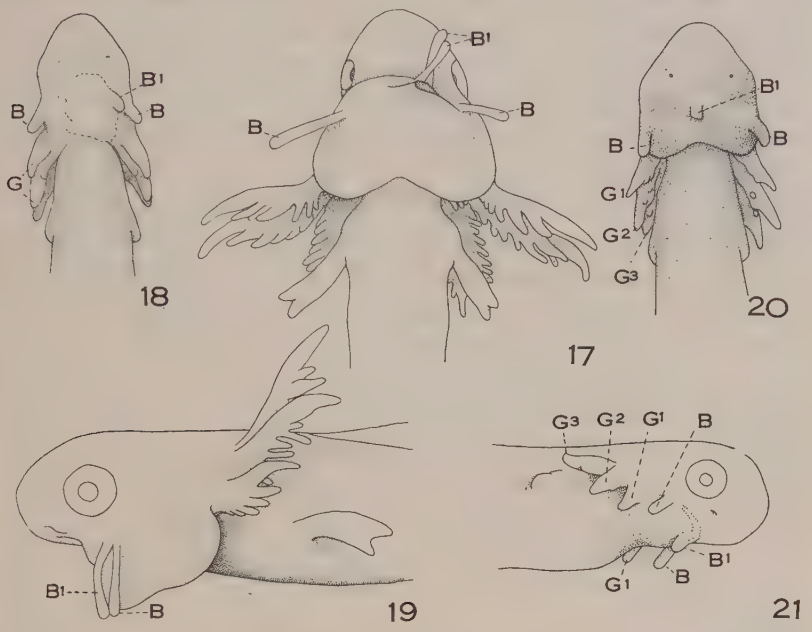
In the first class the placement of the graft was faulty, permitting free ingress of the mesenchyme into the epithelial evagination. Two of these cases have been examined in sections.

The first individual[48] healed well, but the balancer developed a little behind the normal position of the stomodeum and was double (fig. 17, *b'*). Both parts grew rapidly and, together with the deformed lower jaw, gave the embryo a very grotesque appearance. Just before the complete resorption of the yolk (stage 45) the specimen was preserved and cut into transverse sections. Both of the balancers are fully expanded, containing blood vessels and a normal amount of mesenchyme and having a well-developed basement membrane. They are separate where they leave the surface of the head, but where they cross one another, they are united by fusion of the epithelial covering. At the end they are again free. The two balancer membranes are united proximally into a long elliptical funnel, which is attached to an articular cup belonging to an anomalous cartilaginous formation lying under the diencephalon. This process simulates the normal articular cup of the palatoquadrate.

In the second individual of this class[49] the balancer derived from the graft grew out on the left side immediately lateral to the normal position of the stomodeum (figs. 18 and 19, *b'*). It also expanded fully and came to lie close to the normal left

[48] Exp. BSt.1.
[49] Exp. BSt.11.

balancer (*b*). Sections show that the funnel portions of the basement membrane of the supernumerary and the normal balancers are fused together into a single structure, the base



Figs. 17 to 21 Cases showing balancers which have grown in the stomodeal region. *B*, normal balancer; *B'*, grafted balancer; G_1-G_3, gills. × 10.

Fig. 17 Case in which a double balancer developed from grafted ectoderm, taken in stage 25, and free from mesoderm and mesectoderm. Exp. BSt.1, thirteen days after operation.

Fig. 18 Embryo at stage 37 +, showing grafted ectoderm and supernumerary balancer near the normal left balancer. Exp. BSt.11, five days after operation.

Fig. 19 Same individual, fifteen days after operation, showing the two balancers, normal and supernumerary, side by side. Note the distortion of the lower jaw and operculum, due to the failure of the mouth to break through.

Fig. 20 Exp. BSt.15. Embryo at stage 38, five days after operation, showing grafted balancer in collapsed condition lying directly over mouth.

Fig. 21 Same embryo four days after operation viewed from the side.

of which is considerably drawn out in one direction. This is attached to the cup-like articular process of the palatoquadrate, which is likewise drawn out to correspond to the form of the membrane. The cartilages of this region are

very much modified. There is a thick plate extending across from one side to the other under the diencephalon, and this is ankylosed with an anterior extension of the palatoquadrate on the left side and on the right with Meckel's cartilage. The palatoquadrate of the right side is normal, but that of the left is distorted anteriorly, as shown by its elongated balancer cup and by the anterior process which is ankylosed with Meckel's cartilage. The balancer has well-developed blood vessels, but no nerve.

The second class of individuals, those with irregular or collapsed balancers, show very plainly the effect of the exclusion of mesenchyme, confirming the results obtained by grafting the balancer rudiment of an advanced embryo upon a much younger one, as described below (p. 397). All of these operations were done upon older embryos, i.e., just before the balancer made its appearance. The placement of the graft could therefore be determined with much greater precision than in the younger stages, so that when the balancer did appear it was directly over the area where the endoderm was in contact with the ectoderm (fig. 20).

The four cases[50] are closely similar to one another. The balancer itself viewed externally is seen to be short and irregular with its walls collapsed (figs. 20 and 21 *b'*). Sections show that the epithelial cells are very irregular in arrangement (fig. 65). Instead of a single cylindrical cavity filled with mesenchyme, there are a number of irregular cavities (*b.c*) in the epithelium, which may communicate with one another. At the base there are also channels leading to the mesenchyme-filled spaces of the head, through which a few cells (*m.e*) may enter these cavities. It seems almost impossible to prevent this entirely. The cavities are lined by the basement membrane (*b.m*) characteristic of the balancer, which, however, is very much thinner than usual. In some of the cavities there is a network of fibers, of the same substance as the membranes, extending across the spaces.

[50] Exp. BSt.15, 16, 17, and 18.

In the third class of cases[51] the balancer appeared as a nodule, but underwent no further development.

The results of this group of experiments, taken as a whole, show clearly the influence of the substrate in the development of the balancer. When the mesenchyme is excluded or much reduced, the epithelium nevertheless evaginates by virtue of the activity of the ectodermal cells, themselves, but there is nothing to hold it tense and it never fully expands nor do its cells assume their regular arrangement. The basement membrane is likewise affected, and is only incompletely formed, showing that it is the mesenchymal ground-substances that furnish the material for this structure.

Effect of covering the balancer region with strange ectoderm

Just as the experiments described in the previous section show by a positive proof the specificity of the balancer ectoderm, so the following ones give a negative demonstration of the same fact. When the ectoderm is removed from the balancer region and is replaced by ectoderm from other parts of the head or from the body, then the development of the balancer is suppressed.

The grafted ectoderm in these cases was derived as follows: five cases from the branchial region,[52] three from the auditory region and the upper part of the gill swelling,[53] and six from the trunk.[54] The development of the external form in one of these individuals is shown in figures 22, 23, and 24, from which it is seen that the gills are quite normal, but the balancer is absent and the eye defective.

In only one case[55] out of the fourteen was there any trace of a balancer. This individual was grafted in stage 22. Four days later a nodule of considerable size showed in the balancer region. This developed into a rudimentary gill-like appen-

[51] Exp. BSt.2 and 14.
[52] Exp. BrA.2, 7, 8, and 9 and BrEC.8.
[53] Exp. AVBrC. 1, 2, and 3.
[54] Exp. FlEC.8, 10, 12, 13, 14, and 15.
[55] Exp. BrA.2.

dage, of which the embryo had two in addition to its three normal gills, one on the hyoid and one on the mandibular arch.[56] Nothing further in the nature of a balancer that was visible from the outside developed in this case. Sections show, however, that a small fragment of the balancer membrane is present, and that there is a well-developed articular process on the palatoquadrate cartilage. There is no marked abnormality in the form of the palatoquadrate that can be made out from the sections. In the other cases there is nothing to say with regard to the balancer itself, except that it did not develop and that no trace even of the basal portion of the membrane was found. In two of these cases,[57] however, there are indications of the articular process for the balancer on the palatoquadrate cartilage, the cartilage itself being abnormal in form. In one individual the cup is fairly well developed, but arises from near the posterior angle of the palatoquadrate, while in the other it is very rudimentary. Two other cases,[58] preserved at about stage 42, failed to show a definite articular cup, though normally it may be made out in this stage. The remaining cases were preserved too early to show the form of the palatoquadrate. Other cases bearing upon this question will be considered in several of the subsequent sections.

Effect of removal of balancer ectoderm without replacement—regeneration

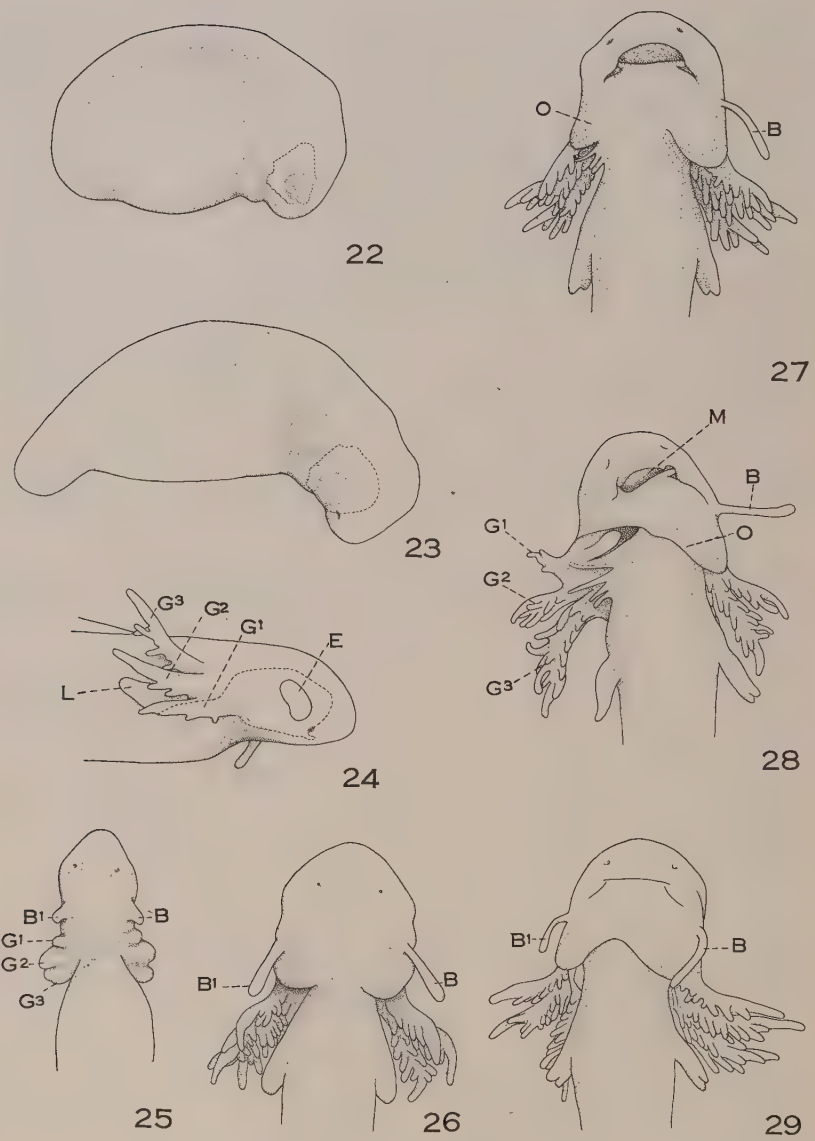
Bell has shown for Diemyctylus that when the ectoderm of the balancer region is removed shortly before the appendage appears, it nevertheless develops, though somewhat retarded. If the ectoderm is not removed until immediately before the time when the balancer would normally bud out, then the appendage developing from the regenerated ectoderm is very small.

[56] Cf. Harrison ('21), p. 165 and fig. 6.
[57] Exp. AVBrC.1 and 3.
[58] Exp. FlEC.14 and 15.

In the present study no experiments have been directed particularly toward the purpose of determining either the exact area of ectoderm capable of producing a balancer or the area that normally forms it. In the absence of very definite landmarks and with the occurrence of a marked shifting of skin areas in the early stages of development, this would be a tedious undertaking. Nevertheless, a considerable number of experiments, made primarily for other purposes, confirm and extend the results obtained by Bell, showing that while the area of ectoderm from which the balancer may develop is limited, it is larger than that which normally enters into this structure. In other words, the ectoderm surrounding the area which normally gives rise to the balancer has the power of replacing the latter, and of regenerating or 'post-generating' the appendage, if the rudiment is removed.

In most of the experiments in which the gill region was covered by ectoderm from other parts of the head, the tissue removed for grafting included the balancer rudiments. Few of the donor embryos in these experiments lived, however, because in many cases they were very young at the time of operation and the denuded areas were so large that the wounds did not readily heal. Only three such cases are available, and out of these the balancer failed to develop in two, and a very small one was formed in the remaining one.

The experiments in which smaller areas were removed were mostly those made for the purpose of transplanting balancer ectoderm from one place to another. Many such individuals also died, but records are available in eighteen cases, in six of which the balancer regenerated and in twelve of which it did not. Only those cases are considered in which balancers developed in the recipient from the grafted ectoderm, for only in these was it certain that the normal balancer rudiment had been removed from the donor. In these experiments, then, two balancers developed from one rudiment, one from the transplant and one from the surrounding ectoderm of the donor.



Figs. 22 to 29 Experiments showing the effect on the development of the balancer of removal of ectoderm or mesoderm from the mandibular region. *B*, balancer on normal side; *B'*, balancer on side of operation; *E*, eye; G_1–G_3, gills; *L*, limb; *M*, mouth; *O*, operculum; outline of graft shown by broken line. × 10.

Sections of these specimens show interesting relations with regard to the balancer membrane and its attachment. In one case[59] with large initial wound, the small balancer which developed articulated with the palatoquadrate cartilage in normal manner, except that the articular cup was proportionately reduced in size to accommodate the smaller basal funnel of the balancer. In the two other cases[60] with large wounds no trace of the balancer was present either externally (fig. 28) or internally, and correspondingly there is no articular cup on the palatoquadrate.

Similar conditions are shown in the cases with smaller wounds. One individual[61] with well-developed balancer about two-thirds normal length (fig. 29) has a full-sized basal funnel joined to a normal articular cup. Two other cases[62] in which the balancer failed to develop (fig. 27) show no trace of funnel

Fig. 22 Embryo from which ectoderm was removed from eye and mandibular region and replaced by ectoderm from the flank. Exp. F1EC.13; operation at stage 19 (medullary folds approximating). Figure shows embryo in stage 23, one day after operation.

Fig. 23 Same embryo, three days later (stage 32).

Fig. 24 Same embryo, eight days later (stage 40) or twelve days after operation, showing the suppression of the balancer. The gills are normally developed, but the eye defective.

Fig. 25 Embryo from which the entire mesoderm of the mandibular arch was removed at stage 22, and the ectoderm healed back. Balancer normal with very slight retardation. Exp. EMM.2, four days after operation.

Fig. 26 Same embryo, eleven days after operation.

Fig. 27 Case in which balancer failed to develop on right side after removal of the mandibular ectoderm alone; denuded area relatively large. Exp. BSt.11—; from the preserved specimen, fifteen days after operation (stage 43).

Fig. 28 Case similar to last except that a still larger area of ectoderm was removed; eye and operculum defective. Exp. CAVBr.1—. Specimen preserved fourteen days after operation.

Fig. 29 Case in which balancer developed, after removal of mandibular ectoderm, from tissue which grew in from side and covered wound. Exp. TrB.16—. From specimen preserved twelve days after operation. A balancer developed also from the removed ectoderm, which was implanted on a Tigrinum embryo (fig. 38).

[59] Exp. ABr.2—.

[60] Exp. CEBr.7— and CAVBr.1—.

[61] Exp. TrB.16—.

[62] Exp. BSt.11— and BSt.12—.

and no articular cup whatever. The palatoquadrate cartilage is itself small in both of these cases and in one is ankylosed with the hyoid.

Effect of amputation of balancer

The balancer was amputated at the base in nine cases in stages 37 (fig. 2) and 39 (fig. 3). No regeneration followed, which confirms the results of Bell's experiments. Four of the cases studied in transverse sections are interesting in connection with the formation of the basal funnel and the articular cup.

In an individual[63] preserved ten days after the balancer was removed in stage 39 the basal funnel is fully developed, projecting into the ectoderm, which covers it over as a thin layer of cells. The blood vessel in the funnel is gorged with corpuscles and the nerve cannot be made out. The articular cup of the palatoquadrate is normally formed and enters into the usual relations with the funnel membrane. The presence of the basal part of the balancer membrane was to have been expected in this case, since it is already well defined in stage 39 and was probably not removed by the operation.

At stage 37 the basement membrane of the balancer is still very thin, and does not extend appreciably beyond the base of the appendage. Correspondingly, specimens in which the balancer was removed at that stage show little or no evidence of the membrane when preserved ten days later. Thus in two cases[64] it was entirely absent at stage 44, while in one[65] it is present in the form of a thin shell having its convex surface toward the ectoderm. This is attached to a poorly developed articular process on the palatoquadrate. The cup is not composed of round cartilage cells, but of flattened cells more like those of the perichondrium, and it resembles the articular processes that are undergoing regressive changes after the balancer is shed. The two cases lacking all traces

[63] Exp. RBal.1a.
[64] Exp. RBal.2a and 2c.
[65] Exp. RBal.2b.

of the balancer show the articular cup in a similar but even more rudimentary condition.

Effect of removal of the mesoderm of the mandibular arch

A few experiments were made to determine the effect of the removal of the mandibular mesoderm. This was done by laying back an oval flap of ectoderm and scraping out the underlying mesoderm before the mesectoderm surrounds it. Six embryos were operated upon just after closure of the medullary folds, in which stage the mandibular mesoderm is clearly segmented off from the rest of the layer. Only two individuals lived sufficiently long to show definite results, and in both of those the balancer developed. In one case[66] it was slightly retarded at first (figs. 25 and 26), but in the other not even this effect was noted. Three more operations, two of which were successful, were done at older stages (stages 24 and 27). Balancers developed normally without retardation in both cases. In the older of these and to some extent in the younger, the mandibular mesectoderm from the ganglion crest must also have been removed.

These experiments, though few in number, show that there is nothing specific in the mesoderm of this region that has to do with the formation of the balancer. This is as expected, since the mesenchyme of the balancer is derived from the mesectoderm, which comes from the neural crest.

Effect of removal of the source of the mesectoderm

As is now definitely known, particularly from the work of Landacre ('21) and Stone ('21 and '22), the neural crest grows down over the side of the head and wraps itself around the mesodermal core of the visceral arches, including the hyoid and the mandibular, giving rise ultimately to Meckel's cartilage, the palatoquadrate, and most of the rest of the visceral skeleton. Before this takes place the balancer grows out and into the evagination a few of these mesectoderm cells push.

[66] Exp. EMM.2.

Doctor Stone has kindly permitted me to examine a number of his specimens in which the whole or part of the neural crest had been removed. In all of these the balancer developed normally, showing that, like the mesoderm proper, the mandibular mesectoderm is non-specific with respect to the formation of this appendage.

Direction of outgrowth of the balancer

Normally the balancer grows out in a ventroposterior direction, its axis, projected on the sagittal plane, making an angle of about 45° with the longitudinal axis of the body. Is the direction of outgrowth determined by the balancer ectoderm itself, by its organic environment, or by a combination of the two factors? The few experiments that have been made point to the last-named possibility. When the balancer ectoderm is inverted, i.e., rotated 180°, the balancer grows out in an abnormal direction, but not dorso-anteriorly, which is the direction it should take if determined wholly by the ectoderm itself. In two cases of this kind the organ grew out more sharply to the side than is normal, but in one it pointed slightly posteriorly and in the other ventro-anteriorly. Other cases in which larger areas were transplanted indicate also that the direction of outgrowth may be changed by change of orientation of the ectoderm, but they are not sufficiently clear to show just how much depends on the orientation of the ectodermal elements and how much upon surrounding factors.

The time factor in differentiation—transplantations between embryos in markedly different stages of development

In order to test the limitation, as regards time, of the power of the ectoderm to form the balancer, experiments were made in which ectoderm of older embryos was grafted to younger ones and others in which that of younger embryos was placed on older ones. These both show that the balancer begins its development at a time which is determined by the age of the grafted ectoderm or, in other words, that the period in which

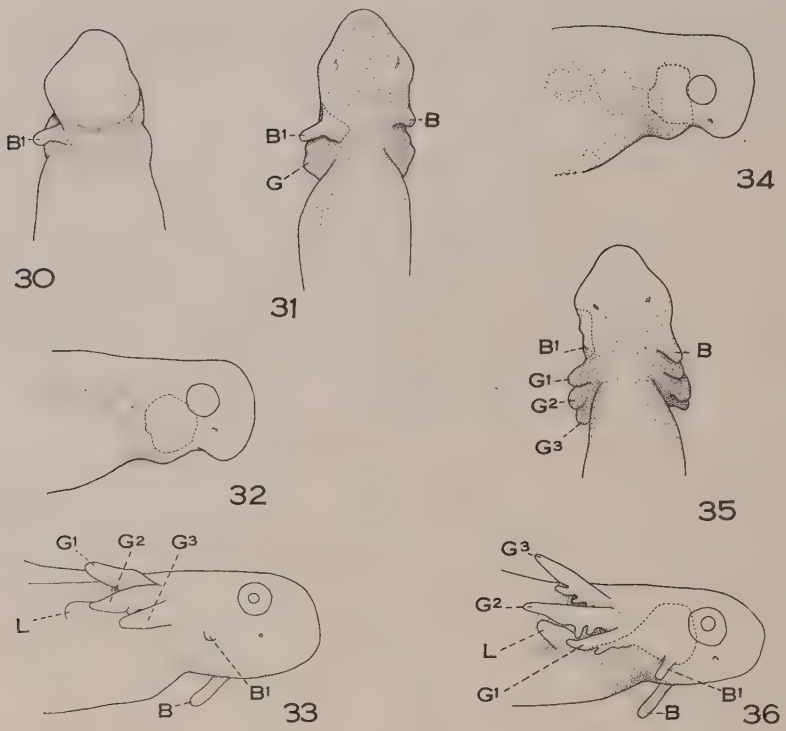
the epithelium is capable of forming a balancer lies within narrow limits. The velocity and completeness of development are, however, influenced to some extent by the subjacent tissue.

Transplantation of balancer ectoderm from older to younger embryos. Such transplantations were carried out in eleven cases, eight of which lived. The donor embryos were taken just prior to the appearance of the balancer (about stage 33), while the recipients ranged between stage 20 and 22 (either just before or just after complete closure of medullary folds). These stages are from three to three and a half days apart at ordinary room temperature of 21°C.

When transplantations of this character are made, the balancer very soon begins to grow out. Usually on the next day after operation it has already become distinct, although the embryo that bears it is not beyond stage 26 or 27. Only in one case out of the eight did it fail to show itself externally. The appearance of the grafted balancer is not, however, normal. It looks more slender and collapsed, like those attached in the stomodeal region (p. 385), where free ingrowth of the mesenchyme is hindered (fig. 30).

Sections of such individuals preserved two or three days after operation[67] show that there is practically no mesenchymal core to the grafted balancer (fig. 61). The main mass of the organ is epithelium, but there are usually several vesicular spaces (*b.c*) surrounded by epithelial cells, which may be connected with one another by smaller canals. These spaces are lined and in some places filled by a fine reticular membrane (*b.m*), which is colored intensely blue in Mallory's stain—obviously an abortive balancer membrane which has failed to develop normally through lack of mesenchymal ground-substance. In one case there are several mesenchyme cells in the balancer. These contain large yolk granules characteristic of the mesoderm proper. They probably entered the balancer before the mesectoderm grew down from the ganglion crest. In another case no mesenchyme cells

[67] Exp. TrB.24 and TrB.25.



Figs. 30 to 36 Showing the effect on the development of the balancer of transplanting ectoderm from older or younger embryos. *B*, normal balancer; *B'*, balancer of side of operation; G_1-G_3, gills; *L*, fore limb; broken line, boundary of graft. × 10.

Fig. 30 Case in which ectoderm from an embryo in stage 33+ was grafted upon one in stage 22. Exp. TrB.24. Individual with a distinct but collapsed balancer (two days after operation). It is at stage 30, which is some time before balancer normally appears; donor at stage 36+.

Fig. 31 Case similar to last. Exp. TrB26. Figure drawn four days after operation. In this case the balancer evagination was filled out with mesenchyme. Embryo now in stage 35+, as indicated by normal balancer on left side of embryo (right of figure), and donor in stage 39—.

Fig. 32 Case in which balancer ectoderm from an embryo at stage 19 (before closure of medullary folds) was grafted to the corresponding region of an embryo at stage 34. Exp. TrB.31. Figure, drawn one day after operation, shows no sign of balancer, which normally should be present as a nodule.

Fig. 33 Same embryo, from the right and below, in stage 38+, four days after operation, with well-developed balancer on normal side and only a nodule on the operated side.

Fig. 34 Similar case to the foregoing. Exp. TrB.29. Embryo in stage 35, one day after operation, with no sign of balancer on operated side, but with incipient bud on the normal.

whatever are present, the balancer being an almost solid mass of epithelium with traces of the blue-staining reticular membrane only here and there.

The oldest specimen examined[68] was preserved four days after the operation, when it had gone a little beyond stage 35, at which period the balancer shows normally as a distinct nodule. The donor embryo had almost reached stage 39 and had a long balancer on the unoperated side. The contrast between the grafted and the normal balancer was strikingly shown in the living specimen just before preservation (fig. 31). This is the only case in which, apparently, sufficient mesenchyme entered to prevent collapse of the evaginating process. The grafted balancer which is placed slightly posterior to its normal position had reached the condition usually attained in stage 37; i.e., it was considerably further advanced than the normal balancer on the opposite side, but not so far as it would have been had it not been transplanted. While it began its development at the proper time, as measured by the age of the grafted ectoderm, its development has obviously been somewhat delayed by the unreadiness of the subjacent mesenchyme to fill out the evagination. The mesenchymal core is more dense than usual and consists, in part at least, of true mesodermal cells, as shown by the large yolk granules. The membrane is very distinct.

Transplantation from younger to older embryos. In this series ectoderm from embryos ranging between stage 19 and stage 21 was grafted upon individuals at stage 33 or 33+. Here the development of the balancer is delayed in accordance with the age of the grafted ectoderm. Seven experiments

Fig. 35 Ventral view of same embryo three days after operation. Stage 37—. Distinct balancer on normal side, but still no sign of it on the side of the operation.

Fig. 36 Similar case. Exp. TrB.44. Balancer ectoderm from an embryo at stage 20 grafted to corresponding region of an individual at stage 33. Figure drawn seven days after operation, showing long balancer on the left (normal) side and much shorter one on the right (side of operation). Embryo in stage 39+; control of same age as donor in stage 38.

[68] Exp. TrB. 26.

were made. In one a small balancer developed early from the ectoderm of the host, the graft having been misplaced. In another case the embryo was killed early, before the time when the balancer should have appeared on the operated side, though it was present on the normal side. In all the remaining cases the balancer did not appear until the donors had reached stage 35, at which time the individuals serving as hosts had reached stage 37 or 38. Sections show in these cases a normal internal arrangement of parts. The youngest individual[69] (figs. 34 and 35), preserved three days after operation just before reaching stage 37, when the normal control, of the same age as the donor, had not quite reached stage 33, has a well-developed normal balancer, but none on the operated side. The grafted ectoderm can be readily made out in sections, however, by its thickness and by the yolk content.

Another case[70] (figs. 32 and 33), preserved at stage 39 six days after the operation, has a grafted balancer normally formed for a stage between 36 and 37, and slightly less developed than the intact balancer of the donor. In sections the membrane is distinct, and the mesenchymal core is rather dense, but the nerve may be followed nearly to the tip. Blood vessels are also present.

A third individual[71] was preserved at stage 40, with the control of the same age as the donor, a little beyond stage 38. Both balancers are well developed; the grafted one closely corresponds to the stage of the control, but the membrane is much thinner than usual. It is provided with blood vessels and the nerve may be traced to near the end. The articular cup of the palatoquadrate on the normal side is well-developed, having reached the procartilaginous stage, but on the operated side this tissue is evidently affected by the retardation of the balancer, for although there is some condensation of the mesenchyme extending out from the quadrate, it is much less differentiated than on the normal side.

[69] Exp. TrB.29.
[70] Exp. TrB.31.
[71] Exp. TrB.49.

The oldest individual[72] of the series was preserved at stage 42, the control being then in stage 40. As in the others, the balancer on the operated side first appeared at stage 37, at which time the control was in stage 35. It is shown at stage 39+ in figure 36. In sections the grafted balancer is seen to be attached slightly in front of the normal position and to be nearly opposite the proximal end of Meckel's cartilage. In consequence of this, a considerable part of the rim of the balancer membrane is not supported by the articular cup of the palatoquadrate, and the posterior part of the latter does not articulate with the balancer membrane. There is, however, an indication of a mesenchymal condensation around the free portion of the rim of the membrane, and the posterior (free) part of the articular cup is not quite so expanded as on the normal side. It is probable that, had the embryo lived longer, some further adjustment of the cup would have taken place. The usual blood vessels and nerve reach the balancer in spite of its displacement. The normal appendage has more secretion cones at the tip than the transplanted one.

Heteroplastic grafts of balancer ectoderm

The strains of Amblystoma tigrinum used in the present investigation (p. 350) have no balancers. It was interesting, therefore, to ascertain what happens when balancer ectoderm of A. punctatum is grafted upon the mandibular region of A. tigrinum and vice versa. The result may be stated in a word: a balancer will develop from punctatum ectoderm growing upon a tigrinum embryo, but no balancer develops when the proper ectoderm of a punctatum embryo is replaced by similar ectoderm from tigrinum; i.e., the development of the balancer is dependent upon the presence of balancer ectoderm from A. punctatum.

Ectoderm from A. punctatum grafted upon A. tigrinum.[73] Twenty-seven experiments of this character were made,

[72] Exp. TrB.44.

[73] I am under great obligation to Dr. W. C. Allee and Dr. C. P. Sigerfoos for their kindness in sending living tigrinum eggs. The present experiments were

seventeen of which yielded definite results, the others dying before the time when the balancer would normally have developed. The age of the donor embryos varied from stage 24 to stage 29, that of the hosts from stage 23 to stage 30, apparently without prejudice to the result, for the appendage developed after operations at both extremes of age.

Out of the seventeen successful cases a balancer (figs. 37 to 41) developed in all but one. In four individuals it was considerably smaller than the normal punctatum appendage,

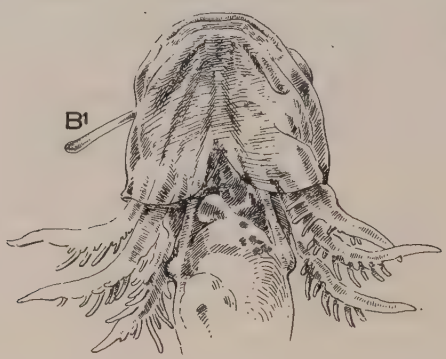


Fig. 37 Larva of A. tigrinum with balancer (*B'*) developed on the right side out of ectoderm transplanted from the mandibular region of an embryo of A. punctatum. Larva with completely resorbed yolk fourteen days after the operation. Exp. TrB.84. × 10.

but in the other twelve it grew almost to the normal size. Circulation of the blood was established in the grafted balancer in at least five cases, in others apparently not; but a negative observation in this matter must not be taken as conclusive that no blood ever circulated in the organ. A nerve from the trigeminus, the normal source of the balancer nerve in A. punctatum, has been found in some of the individuals.

Seven cases have been examined in serial sections. The youngest of these[74] was preserved at stage 38, at which time

made with material collected by Doctor Allee in the vicinity of Chicago, Illinois, except in a few cases in which eggs laid in the laboratory by axolotls collected in North Dakota were used.

[74] Exp. TrB.74.

the control (substituted for the donor) was in stage 37. The grafted balancer had, however, fallen a little behind in development, not being quite so long as that of the control. Oblique sections, cutting the balancer lengthwise, show that its structure is normal, with the regular columnar inner and cuboidal outer layer of epithelium. The basement membrane is thinner than in the normal A. punctatum of stage 37. Blood vessels are present; the arterial connection with the hyomandibular artery can be made out, as in the normal punctatum embryo, but the venous connection is uncertain. There is a nerve present, which, however, could not be traced to its central connection.

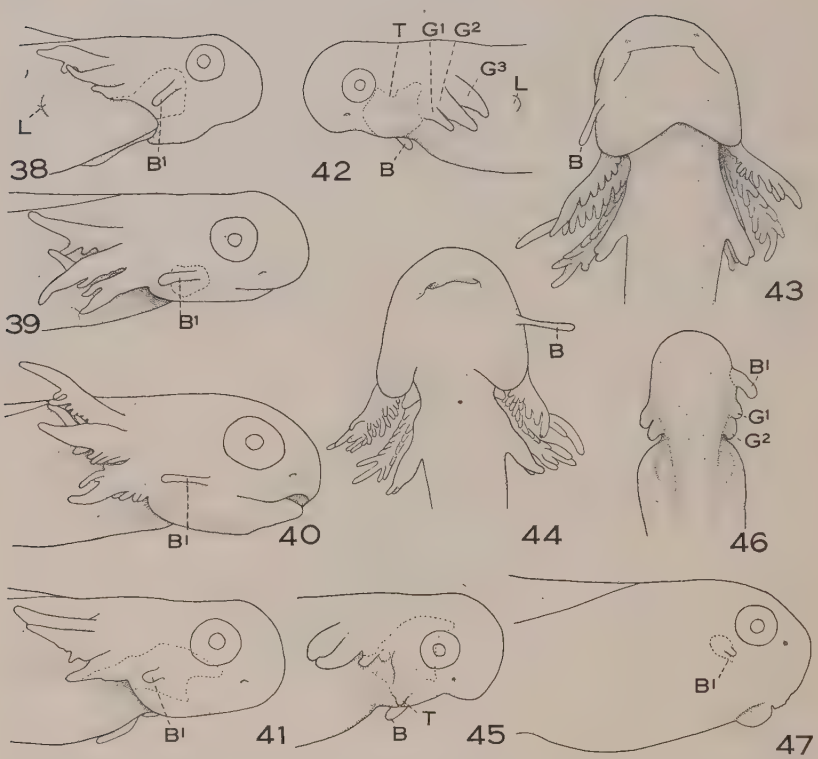
In the next individual[75] the balancer bud likewise appeared at the proper time, but it remained slightly behind that of the control in length (fig. 41), until the specimens were preserved seven days after the operation. At this time the tigrinum embryo was approximately in stage 40[76] and the control a little beyond stage 39. Sections were again cut obliquely, parallel to the long axis of the balancer. The organ is quite normal looking for one of stage 38–39. The blood vessels, both artery and vein, are normally placed and have normal connections. The nerve is also very plain. The balancer is attached opposite the articulation between palatoquadrate and Meckel's cartilage or slightly anterior to its normal position. There is a process of more concentrated mesenchyme running out laterally from the palatoquadrate, but it is scarcely definite enough to be identified as an articular cup, which even in normal punctatum embryos of this stage is scarcely more than a mesenchymal thickening.

In the third case[77] the balancer developed well from the beginning, but nevertheless lagged behind that of the donor. Eight or nine days after the operation (fig. 38) secretion

[75] Exp. TrB.50.

[76] The stages in the development of A. tigrinum have not been so definitely established as those of A. punctatum. The later stages prior to complete resorption of the yolk are passed over rapidly and, owing to the rudimentary character of the fore limb, are difficult to define.

[77] Exp. TrB.16.



Figs. 38 to 47 Results of heteroplastic grafting. *B'*, grafted balancer; G_1–G_3, gills; *L*, fore limb; *T*, tubercle; broken line, boundary of graft. × 10.

Fig. 38 Exp. TrB.16. Amblystoma tigrinum embryo with balancer developed from punctatum ectoderm grafted in stage 25. Ventrolateral aspect; sketch made eight days after operation.

Fig. 39 Similar case, exp. TrB.17, eight days after operation.

Fig. 40. Same individual. Figure drawn from specimen preserved thirteen days after operation.

Fig. 41 Similar case, exp. TrB.50, six days after operation; grafted in stage 26.

Fig. 42 Experiment TrB.20. Embryo of A. punctatum with ectoderm of balancer region replaced by corresponding ectoderm from A. tigrinum, drawn six days after operation at stage 38; development of balancer suppressed; *T*, position of rudimentary balancer funnel.

Fig. 43 Same case, ventral view, drawn from specimen preserved twelve days after operation.

Fig. 44 Similar case, exp. TrB.77. Balancer suppressed on operated side, operculum slightly defective; drawn from specimen preserved sixteen days after operation.

cones were very distinct at the tip, and two days later movement of blood in the balancer vessels was plainly visible. The specimen was preserved at twelve days (about stage 45). The grafted balancer is considerably shorter and more slender than that of the donor, but it points normally and is attached at the proper place. Transverse sections (fig. 59) show that it is in normal position with respect to the palatoquadrate cartilage (*p.q*), from which there is a condensation of mesenchyme (*a.p*) running out toward the basal funnel (*b.f*). This is not, however, so definite as in the normal punctatum larva, though more so than anything which might correspond to it on the unoperated side of this tigrinum individual. It looks as if the presence of the grafted balancer had stimulated its development in this case. The funnel is well-embedded in the mesenchyme, but there is no close attachment to the process which runs out from the palatoquadrate. A nerve twig from the trigeminus may be traced into the appendage.

Another case (figs. 39 and 40)[78] is much like the preceding, except that the balancer is not quite properly placed with respect to the palatoquadrate cartilage. There is some condensation of mesenchyme running out from the anteroventral angle of this cartilage toward the rim of the balancer membrane, but it is less definite than in the preceding case.

Mandibular ectoderm from A. tigrinum transplanted to the balancer region of A. punctatum. Twenty-six experi-

Fig. 45 Similar case, exp. TrB.58, five days after operation. In this case a nodule (*T*) developed in the lower part of the mandibular region. Sections show that it contains a tooth germ and that it has nothing to do with the balancer, which is completely suppressed.

Fig. 46 Dorsal view of an embryo of Rana sylvatica to which balancer ectoderm from an Amblystoma punctatum embryo (stage 34) had been grafted. Exp. TrB.28, two days after operation, at which time donor had reached stage 36+.

Fig. 47 Similar case. Ectoderm from A. punctatum grafted in stage 33 on R. sylvatica. Exp. TrB.34, seven days after operation. The grafted balancer is atrophic.

[78] Exp. TrB.17.

ments of this kind were made, but owing to the high death rate only twelve were available for study. The balancer developed in three of these and was suppressed in the remaining nine. In the three former cases, however, the balancer ectoderm of the host was not entirely removed and the graft consequently not properly placed, the balancer developing from the ectoderm of the host. This was shown clearly by means of the contrast between the cells of the graft, which were stained intra vitam in Nile-blue sulphate,[79] and the brown pigmented ectoderm cells of the host. The nine cases in which the balancer failed to develop show that the embryo is unable to form this organ without the specific ectoderm of A. punctatum. They supplement the reciprocal experiments of the previous series by adding a negative proof to the positive one given by the latter.

A number of these cases have been examined in sections, but with a single exception there are no internal signs of a balancer. One case[80] (fig. 45) is interesting because of the development of a nodule within the grafted area closely resembling a balancer bud. Sections showed, however, that this excrescence contained a tooth germ and was obviously a misplaced portion of the dental ridge. There was no suggestion of a balancer membrane.

Specimens preserved at later stages indicate that the articular cup of the palatoquadrate is much reduced when the balancer is absent. One case[81] at stage 42 shows only a condensation of mesenchyme instead of a cartilaginous process. In another individual[82] preserved between stage 43 and 44 (fig. 44) the articular cup is likewise lacking, only a few flattened cells being present in its place. The basement membrane underlying the grafted ectoderm is thicker than in other places and in places is separated from the epithelium by a small amount of reticulum.

[79] Cf. Detwiler ('17).
[80] Exp. TrB.58.
[81] Exp. TrB.68.
[82] Exp. TrB.77.

In one of the cases[83] in which the balancer did not develop a nodule was observed just over the boundary of the graft between the eye and gills and a little dorsal to the normal position of the balancer. This was first seen five days after the operation (fig. 42, *t*), but it developed no further. Sections of the specimen preserved a week later (fig. 43) show a distinct balancer funnel embedded in the mesenchyme and attached partly to a rudimentary articular cup on the palatoquadrate. Unlike the basal funnels found after the normal shedding of the balancer (figs. 57 and 58), this structure has no lateral outlet, but is merely a rounded cone of membrane substance. It is undoubtedly identical in position with the nodule noted above and represents an abortive balancer. It was formed under the influence of host ectoderm from a region a short distance outside the area which normally gives rise to the balancer bud, but which still has the potency to produce this organ.

Transplantation of balancer ectoderm from A. punctatum to embryos of Rana sylvatica. Embryonic ectoderm from the mandibular region of A. punctatum was transplanted to the side of the head of Rana sylvatica embryos. In five of the eight cases the donors were in stage 33 or very slightly older and the recipients in stage 20; i.e., just before complete closure of the medullary folds. In three others there was not so much discrepancy in the respective ages of the embryos. These divergent stages were used, as in many experiments upon the gills, because of the far greater rapidity of development of the frog embryo. Previous experiments with lens epithelium had shown that, while Amblystoma embryos are tolerant to Rana palustris ectoderm, the reciprocal combination results in the sloughing of the graft in about two days. With R. sylvatica this reaction is much less intense, and though there is a tendency in this combination also for the frog ectoderm to encroach upon the Amblystoma graft, the latter remains intact at least for some days. The result was an attempt to form a balancer in six of the cases. In two

[83] Exp. TrB.20.

of them only nodules were produced, but in the best a fairly well-marked balancer developed (figs. 46 and 47).

In the first case[84] a process looking much like a normal balancer developed two days after grafting (fig. 46, *b'*), at which time the specimen was preserved. Sections show that the structure is a nearly solid outgrowth of Amblystoma cells with a mesenchyme-filled papilla at the base, and that histologically it has very little resemblance to a normal balancer. It is not unlike the collapsed balancers which develop in the stomodeal region and from grafts upon younger embryos (cf. pp. 385 and 396).

Another embryo[85] showed the day after operation a nodule which two days later grew to a distinct balancer-like process. On the sixth day, when the embryo was preserved, the process was still longer and the boundary of the grafted ectoderm indistinct. Sections show a very abnormal structure. The grafted skin encloses an irregular cavity or cavities, partly filled with mesenchyme and partly vesicular without organized contents. There is a sheet of reticular tissue lining, as it were, the part of the cavity that is filled with mesenchyme, but the vesicular part is devoid of membrane. The mesenchyme cells are clearly from the frog tissue, as may be readily seen from the size of the nuclei. The epithelium is irregular, but the boundary between the cells of the two species is plain in nearly all sections.

In a similar case[86] a well-developed balancer had formed four days after the operation. The following day this outgrowth looked atrophic. Two days later (seven after operation) the embryo was preserved (fig. 47). Histologically, this is the most perfect of the four cases. There is a distinct epithelial tissue consisting of evaginated Amblystoma ectoderm, but the cells are not so regular as those of the normal balancer and show degenerative changes. Inside of this is a distinct balancer membrane, which is not so dense as it

[84] Exp. TrB.28.
[85] Exp. TrB.33.
[86] Exp. TrB.34.

normally is. At the base it expands into a funnel which lies embedded in mesenchyme, and the latter tissue extends a short way into the balancer outside of the membrane, thus separating the latter from the epithelium farther distally than in normal cases. The core of the balancer (inside the membrane) contains many cells, which are clearly of frog origin. No blood vessels can be made out, but a distinct nerve twig from the trigeminus may be seen entering the base of the funnel.

In the last specimen to be described[87] no distinct balancer developed—only a nodule which persisted as such until the embryo was preserved six days after the operation. Sections show the grafted skin to be well marked off from that of the host (fig. 60, *e.b*). There is a distinct balancer membrane (*b.m*) lining the evagination, but its outer portion looks as if it had been eroded by the mesenchyme. The epithelium is columnar. A nerve fiber (*n*) from the trigeminus runs into the small evagination, the cavity of which is occupied by a few mesenchyme cells. No blood vessels are present in the organ.

These cases show that the balancer ectoderm of Amblystoma punctatum, while undergoing degenerative changes consequent upon its implantation upon the frog embryo, is nevertheless capable of reacting with the mesenchymal ground-substance of the latter, and transforming its outer layer into a balancer membrane, as it does normally with the tissue of its own species. It also shows tendency to elongate under these conditions and the evaginating process seems to have the power to trap a nerve fiber as does the normal balancer.

SUMMARY AND CONCLUSIONS

From the foregoing study we may picture to ourselves the development of the balancer taking place as follows: At an early period, probably during gastrulation,[88] the potencies

[87] Exp. TrB.37.

[88] Cf. Spemann ('18).

of the ectoderm are distributed (Driesch) or localized; in other words, differentiation takes place. At first there is no visible sign of the differentiation, which can only be detected by the subsequent behavior of the several regions of the ectoderm under various experimental conditions. During this period the areas that are to give rise to gills, balancer, nose, ear, other placodes, hypophysis, and lens are segregated according to a pattern, not, however, with any very definite boundaries of its several components, but rather in such a manner that the organs just enumerated appear as centers of differentiation, with their respective potencies most intensely active at certain points from which the intensity gradually diminishes peripherally.[89] Intermediate areas, as for example between gills and balancer, may thus, according to the influence under which they fall, give rise to one or the other of the organs involved.

That the factors necessary to call forth the development of the balancer are localized in a certain region of the ectoderm, which ultimately comes to lie over the mandibular arch, is shown by numerous experiments: 1) This area of ectoderm, when transplanted at any time between the medullary plate stage—earlier stages have not been tried—and the period just before the balancer appears externally, will give rise to a balancer on other parts of the head. 2) When implanted upon an embryo of A. tigrinum, this same ectoderm of A. punctatum will bring about the development of a balancer, even though the organ is normally absent in the former species. 3) When the balancer ectoderm is replaced by ectoderm from some other region of the head or trunk, the development of the balancer is suppressed. 4) When the balancer ectoderm of a punctatum embryo is replaced by the corresponding area of a tigrinum embryo, the development of the appendage is likewise suppressed. 5) Removal of either the mesoderm of the mandibular arch or the neural-crest mesectoderm of that region does not interfere with the normal development of the balancer. 6) In transplantations of

[89] Cf. Harrison ('18), p. 456.

balancer ectoderm between embryos of different ages the time of appearance of the balancer is determined by the age of the ectodermal graft and is independent of the age of the embryo which receives it.

For a long period the differentiation of this patch of ectoderm remains invisible. The actual degree of differentiation, however, gradually becomes more and more pronounced, for tissue taken from stages at the beginning of, or just prior to, visible differentiation and transplanted to strange regions behaves in a more specific way than tissue taken at earlier stages. Thus a balancer develops from ectoderm taken from younger embryos only when transplanted to the head, presumably on account of the mesectoderm found in that region. Taken in later stages, just before the appearance of the bud on the surface, the proper ectoderm will give rise to a balancer even when implanted in the trunk region. If the mesectoderm is taken with the ectoderm in younger stages, the balancer will develop also in the trunk region.

The first visible sign of differentiation is a change of form of the ectodermal cells covering the middle of the mandibular arch. The inner layer thickens, its cells becoming columnar; the outer layer changes to a less degree, its cells becoming cuboidal. Then an evagination takes place mostly through a reversed change in shape of the epithelial cells, the two layers becoming thinner through the flattening and superficial extension of the cell bodies. The mesenchyme, derived from the mesectoderm covering the mandibular arch, pushes out into the pocket formed by the evaginating ectoderm (figs. 50 and 51).

At this time the condensation of the mesenchymal ground-substance directly under the evaginating epithelium begins, whereby a continuous basement membrane (*b.m*) is formed. This is very much more sharply defined than the basement membrane[90] elsewhere in the embryo, which, in fact, at this

[90] The term basement membrane has been used here not as designating anything sui generis, but merely because of its topographic relation in underlying the epithelial layer. As pointed out by Merkel ('09, p. 332), this layer, which he calls

early stage can scarcely be made out at all. The balancer membrane is clearly formed in the mesenchyme and is in no sense a secretion of the ectoderm. On the other hand, the cellular elements of the mesenchyme obviously have no direct specific effect upon the formation of this structure. They show, at no time, any definite topographic relation to the forming membrane, which often for long stretches is quite free from mesenchyme cells (figs. 52, 53, and 54). We must, therefore, regard this structure as a product of the action of the epithelial cells upon the underlying undifferentiated mesenchymal matrix, by which the latter is changed from its indefinite diffuse condition to a sharply defined membrane composed of a dense felt of reticulum fibers. It is quite possible that the mechanical effect of the evagination may have something to do with its formation, but this cannot be the sole cause or even a very important factor, since in the external gills, which grow out simultaneously with the balancer, no such membrane is formed, and part of the membrane may be found in cases where no pronounced outgrowth occurs. We must look to some other kind of activity in the epithelial cells, and one naturally thinks of an enzyme action which condenses or coagulates the underlying diffuse intercellular ground-substance[91] transforming it into a fibrillar tissue which gives almost the same chemical reactions as reticulum.[92]

The balancer membrane thus affords a clear proof that connective-tissue fibers take origin in an amorphous ground-substance, independently of any direct action on the part of the mesenchyme cells. The fibers composing the membrane are neither intracellular nor are they formed in any particular outside layer which could be called exoplasmic,[93] unless

the membrana terminans, is everywhere of connective-tissue origin and is never formed under the direct influence of the cells of the connective tissue. Studnička ('07 and '11), Schuberg ('08), and others identify it as the beginning of the corium.

[91] Cf. Nageotte ('16).

[92] Cf. Mall ('96).

[93] Cf. Mall ('02), Hansen ('00, '05).

the whole of the intercellular ground-substance be designated as such.[94] This is in harmony with the view taken by von Ebner ('97), Merkel ('09), Studnička ('07, '11), Nageotte ('16, '22), and by Baitsell ('15, '16, '17, '21) who has traced very carefully the earliest differentiation of connective-tissue fibers in the amphibian embryo and in wound healing. The balancer membrane develops much like the corium in fishes and amphibians, where, according to Studnička, a fibrous layer is laid down beneath the ectoderm, and this is at first devoid of cells, being formed without the direct intervention of cells. The balancer membrane is precociously formed, however, i.e., before the corium appears elsewhere in the embryo, and it is formed very rapidly, becoming very dense and staining most intensely in the anilin blue of Mallory's stain. This reaction is in some way, however, subject to the action of the balancer epithelium which is already localized and differentiated at this period. We have before us an instance of a structure called forth in an indifferent tissue through the influence of a specialized adjacent tissue.

The formation of the membrane goes on while the balancer is rapidly increasing in length. At the same time the outer epithelial layer is also undergoing differentiation, as shown by the modification of its cuticular border, which becomes specialized for the secretion of a mucoid substance—one which, however, does not give the characteristic reaction with Mayer's mucicarmine. The balancer epithelium thus differs from the usual epithelial tissue in that its inner layer is specialized for action in the direction of the substrate, while the outer layer forms a secretion toward the exterior.

The active evagination process is due solely to qualities localized in the ectoderm. However, in order that it may be carried out successfully, it is necessary that a substrate of mesenchyme be in readiness to fill out the cavity produced by the evagination. This is shown by the collapsed and irregular condition of balancers that grow in the absence of such

[94] Cf. Studnička ('11).

support when, for instance, the balancer is grafted upon a much younger embryo, or when it is placed in the stomodeal region, where free access of mesenchyme is prevented. When the latter material is deficient, the basement membrane is also defective. There is some sort of membrane formed, but it is insufficient in quantity, so that the rigidity of the organ is much reduced.

The changes that take place in the ectoderm, resulting in the formation of the balancer, are limited as to time just as they are in respect to space. Ectoderm from an embryo just before the appearance of the balancer, when transplanted to a much younger individual, grows out immediately though the underlying tissues are not ready for it. Conversely, when ectoderm from a young embryo is placed in the proper location upon an individual just about to develop its balancer the development is delayed until the grafted ectoderm reaches the necessary maturity.

At about the time the organ is evaginating the main nerve paths are being laid down in the embryonic body. The mandibular division of the fifth nerve runs not far from the base of the balancer, and from this nerve a twig enters the appendage and may be followed to near the tip. This nerve probably belongs to the general cutaneous system, since no special sense organs can be detected in connection with it. When the balancer is cast off, the peripheral part of the nerve is lost with it, but regeneration takes place, the new nerve innervating the skin in the region of the base of the jaw (fig. 58).

A nerve is found in the majority of cases even when the balancer develops in abnormal position after transplantation of the ectodermal rudiment. The nerve in such cases may arise from the trigeminus, the facial, the glossopharyngeal, the vagus, or from a spinal nerve. It may run in from the r. mandibularis in Amblystoma tigrinum, which normally has no balancer, when it possesses a balancer derived from a punctatum graft. Even when the balancer ectoderm is implanted on the frog embryo, the abortive balancer that

develops may receive a nerve from the host. Clearly, there is some factor directing the course of the nerve fiber into the appendage, but it must be of a general and not of a specific nature.

Normally blood vessels run to the appendage at a very early stage. When transplanted, vessels from other sources may enter, but there seems to be less uniformity about this than in the case of the nerve, and it is difficult to detect movement of the blood even in cases where vessels are present.

As the balancer matures, the basement membrane, which is a long tubular structure, thin at the extreme end but thick and rigid with a funnel-like flange proximally, becomes detached from the epithelium at its base probably by the expansion of the mesenchyme ground-substance, by which action the epithelium is pushed away from it. By pancreatic digestion the membrane may be isolated, and it then appears glass clear, shaped like a long, thin test-tube (fig. 49). In its reactions to digestive ferments, acids, and caustic potash it resembles reticular tissue and not bone.

As the young Amblystoma approaches the time of complete resorption of its yolk, changes are seen to take place at the base of the balancer. The epithelium proliferates, the membrane becomes ragged and is finally broken through, the nerve and blood vessels being cut off by the epithelium at the same time. Then the balancer drops off, leaving the basal funnel embedded in the mesenchyme, where it remains for a long period, perforated by blood vessels and nerve.

When the palatoquadrate cartilage chondrifies, beginning shortly after the balancer funnel is detached from the epithelium, an extension of this cartilage runs laterally to meet the funnel, first as a condensation of mesenchyme cells and later as actual cartilage.[95] The funnel thus becomes attached around its rim to this articular process, or cup, of the palatoquadrate. Two notches are left, however, a ventro-posterior one for the artery and an anterodorsal one for the vein and

[95] Cf. Orr ('88) and Winslow ('98).

nerve. The attachment becomes more and more firm, the cartilage becoming more sharply differentiated, until the balancer is shed. From this time on, however, a reverse process sets in, and the articular cup becomes dedifferentiated into fibrous tissue, its cartilaginous matrix being resorbed and its cells becoming flattened. Later it becomes reduced to a diffuse mesenchyme.

The relation of this structure to the balancer is such that when the latter is lacking, then the articular cup does not undergo its full development. In fact, in many cases it is not developed at all. Some of the cases are difficult to interpret because of the irregular development of the palatoquadrate and its articulation with Meckel's cartilage after transplantations in that region, but out of twelve cases in which the balancer failed to develop, there are only two in which the articular process is at all clear. In some cases it is entirely lacking, while in others it appears merely as a fibrous structure not unlike the condition in which it is found during the regressive changes that normally take place after the balancer is shed.

The dependence of the articular cup upon the balancer is also clearly shown in several cases in which it became adjusted to fit the shape of an abnormally formed funnel—particularly in one in which a grafted balancer was fused proximally with the normal one, forming an elliptical base, and in another such case in which a double balancer developed in the stomodeal region and in which the articular cup was developed de novo upon an anomalous cartilaginous element.

Further positive evidence is given by the behavior of the palatoquadrate in A. tigrinum, which normally lacks the articular cup, in cases where a balancer develops from transplanted punctatum ectoderm. Here a condensation of the mesenchyme takes place in a position corresponding to that of the articular cup in A. punctatum, and this may even become partly chondrified, although never completely developed (fig. 59).

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PLATES

ABBREVIATIONS

a.m, abdominal muscle
a.h, hyomandibular artery
a.p, articular process of the palato-quadrate cartilage for support of the balancer
b, balancer
b.f, balancer funnel or basal part of membrane
b.m, balancer membrane
b.v, blood vessel of balancer
c, cartilage developed from graft
cut, cuticular border of ectoderm
e.b, boundary between tissue of host and graft
e.d, deep layer of epidermis
e.p, epithelial plug cutting off the balancer
e.s, superficial layer of epidermis
m.e, mesectoderm of mandibular arch
mes, mesoderm of mandibular arch
n, nerve of balancer
p.q, palato quadrate cartilage
s.c, secretion cone

The figures are all placed on the plates with the ventral side toward the bottom, except in the case of figure 62, which is part of a frontal section.

PLATE 1

EXPLANATION OF FIGURES

48 Tip of balancer of live specimen of A. punctatum (stage 43), showing the outer layer of flattened epithelium with its cuticular border (*cut*), the vascular loop (*b.v*), and the secretion cones (*s.c*). × ca.200.

49 Complete balancer membrane after digestion of the soft parts in pancreatin. From an embryo, stage 43. × ca.50.

In the specimens represented in figures 50 to 55, inclusive, the plane of section is oblique, as shown by line in text figures 2 and 3, and cuts the balancer longitudinally.

50 Portion of a section through the mandibular region of an embryo, stage 35 (A. punct. 59) showing the beginning of the balancer evagination and the laying down of the membrane. × 195.

51 Similar section from an embryo in stage 36 (A. punct. 53). × 195.

52 Similar section of an embryo stage 38 (A. punct. 61). × 195.

52A Whole section through balancer region, stage 38 (A. punct. 61). Same section as figure 52.

53 Portion of section (cutting the balancer longitudinally) of an embryo in stage 39—(A. punct. 62), showing the proximal end of the balancer membrane and its relation to the ectoderm and the mesenchyme. × 338.

54 Section, similar to the one shown in figure 53, from a slightly older embryo (stage 40, A. punct. 64). × 338.

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PLATE 2

EXPLANATION OF FIGURES

55 Portion of an oblique section through the head of an embryo in stage 41 (A. punct. 66), showing the base of the balancer and the articulation of the membrane with the palatoquadrate cartilage. × 195.

56 Portion of a transverse section through a larva in stage 46 (A. punct. 76), showing the autotomy of the balancer. The fully developed articular cup is also shown. × 195.

57 Similar section, from an embryo preserved just after shedding balancer. Stage 46 (A. punct. 75). × 195.

58 Portion of transverse section through the head of a larva about 16 mm. long preserved about three weeks after complete resorption of yolk (exp. TrE.58—). The funnel-like base of the balancer membrane is still traversed by the balancer nerve, which is now distributed to the skin over that region. The articular cup has been resorbed. × 195.

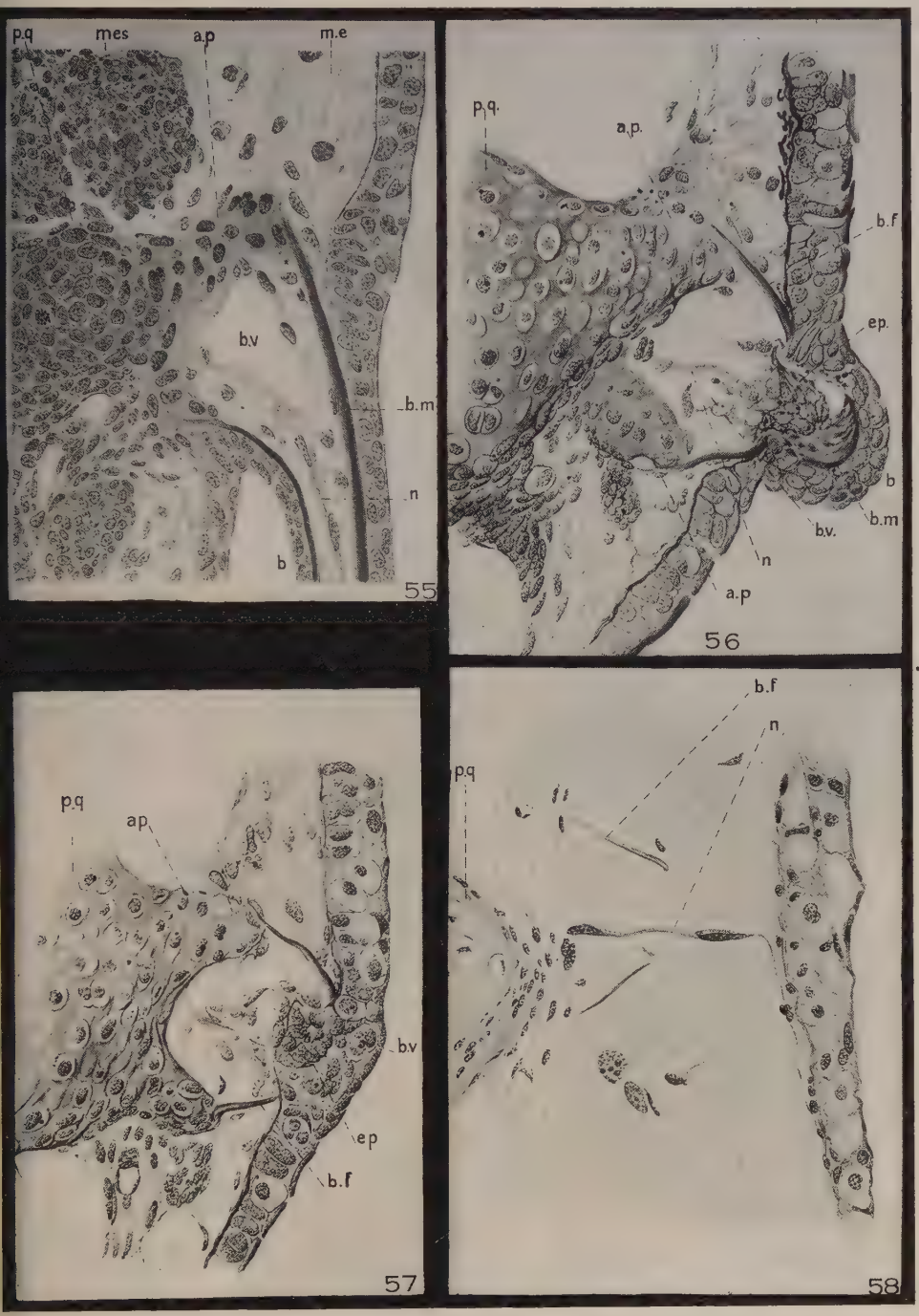

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PLATE 3

EXPLANATION OF FIGURES

59 Section through the base of a grafted balancer, A. punctatum on A. tigrinum (exp. TrB.16), showing the rudimentary articular process of the quadrate not normally found in A. tigrinum. × 195.

60 Section through the base of a grafted balancer A. punctatum on Rana sylvatica. The membrane has been partly separated from the ectoderm. Note the sharp boundary (*e.b*) between the Amblystoma and the Rana ectoderm and the presence of a nerve (*n*). Exp. TrB.37. × 195.

61 Section through a balancer developed from ectoderm of an older embryo (stage 33+) grafted to a younger embryo (stage 22). Specimen preserved three days after operation in stage 33, at which time normally no balancer is developed; donor embryo at that time in stage 37 with well-developed balancer bud. Exp. TrB.25. × 195.

62 Exp. BFl.8. Portion of a frontal section showing the base of the balancer developed from a piece of mandibular ectoderm grafted on the flank. The donor was in stage 32 and no mesectoderm was taken with the graft. × 195.

63 Exp. BFl.17. Portion of a transverse section through the abdomen of an embryo, showing base of grafted balancer. The graft was taken from an embryo in stage 26+ and included both ectoderm and mesectoderm. The latter has given rise to a nodule of cartilage (*c*). The balancer membrane is distinct and a nerve enters the cord of the appendage. × 195.

64 Exp. CEBr.25. Portion of section through region of the second gill, showing the expanded base of a transplanted balancer. The grafted ectoderm was taken from an embryo with wide open medullary folds (stage 17) and included a few mesoderm (not mesectoderm) cells. × 195.

65 Exp. BSt.17. Portion of section through the front part of the head of an embryo with a balancer grafted upon the stomodaeal region, showing the appendage in a collapsed condition, owing to the lack of access of mesoderm. × 195.

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Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 42, No. 1, May, 1925

THE SEX OF PARABIOTIC TWINS IN AMPHIBIA

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SIX PLATES

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1. THE PROBLEM

Introductory

The theory of sex hormones as the primary agents in differentiation of sex was given a definite status by the work

of Lillie on the free-martin, published in 1916 and 1917. Independent work was done about the same time by Tandler and Keller ('16) on the same material, which in the main agrees with the work of Lillie. The evidence from castration and transplantation of the gonads had already strongly suggested this theory, and numerous attempts to modify sex by this means had been sufficiently successful to give the idea a certain vogue.

The work of Lillie and those collaborating with him has given strong evidence that the free-martin is zygotically a female, and that the strong modification of her sexual anatomy, whereby the most fundamental structures of sex have been made to approach decidedly the male type, has occurred as the result of a circulation of the hormones of the male through the body of the female at a relatively early period of foetal life while the developing organs of sex are yet in a plastic state. This cross-circulation is permitted by an anastomosis of foetal vessels. Chapin ('17) and Willier ('21), on the basis of a histological examination of the gonad of the free-martin with reference to structural homologies, have pronounced the organ an ovary that has undergone partial transformation, approaching decidedly the male type. Bascom ('23) showed that in the male the hormone is probably liberated at a much earlier stage of development than is the case in the female, hence the constant dominance ascribed to the male. Gowen's ('22) investigation of sex ratios and the lack of occurrence of identical twins in cattle also supports the view that the free-martin is zygotically a female. Therefore, the case for the hormone as a sex-differentiating agent seems well supported.

The case of the free-martin may be regarded as an example of a natural experiment, in which the necessary conditions for the attainment of the particular result came about by chance in the natural course of development. The chance element lies in the fortuitous connection established between foetal circulations in individuals of different sex. Dominance is established by precedence in the development of one sex,

in this case the male, and the incompleteness of this dominance is satisfactorily accounted for by the fact that the vascular anastomosis necessary for transfusion of the hormone is usually not well established until some time after the beginning of sex differentiation, so that the opportunity of working upon a non-differentiated ground-material is lost. In reapproaching the problem from an experimental standpoint, it is desirable to use material in which this last-mentioned difficulty is removed. The material upon which this experiment is based is adapted to this end, it being feasible to join individuals parabiotically at a very early stage of development.

The conditions exhibited by the free-martin and the accepted cause of these conditions immediately suggest the possibility of a complete transformation of sex if conditions of union between individuals are such that a free interchange of hormones can occur from a very early period of development, before there is any differentiation of sex whatever. Although Lillie has made no actual prediction of this result, his attitude indicates that he considers it quite possible. The same thought apparently has occurred to others, and the question of the possibility of reversing sex by means of a hormone has been proposed.

Method of approach

Obviously, the selection of a suitable method of approach is essential. The method of parabiosis, that is, uniting individuals of different sex, is suggested. This method has been used before with the idea of modifying or transforming sex. Morpurgo ('08) joined rats of different sexes by an operation in which the animals were united side to side so that the abdominal cavities were continuous and the pectoral and abdominal muscles well grown together. He obtained little if any change in the sex characters, and attempts at breeding the male member of the pair with a third rat produced young that were quite normal. Harms ('11) used the method of parabiosis in studying secondary sex characters in frogs. He

found that castrated animals when attached to a normal of the same sex maintained their secondary sex characters and the mating instincts. But in a single case in which a castrated male was attached to a female, the external secondary characters were soon lost, although no further modification occurred.

The appearance of Lillie's work on the free-martin stimulated Yatsu ('21) to use parabiosis as a means of approaching the problem. Young rats ranging in age from 29 to 90 days were joined (manner of joining not described) for periods ranging from 11 to 179 days. There were 24 successful unions accomplished. No considerable modification was produced. There was an abnormal tendency to degeneration of the Graafian follicles in female components of pairs (which, however, also occurred when the male component had been castrated) and frequently a hydrometral condition of the uterus. Practically no effect was noted in the male. The testis and prostate were unchanged. It appears from information furnished by Yatsu, that Matsuyama ('19) had performed almost similar experiments and with the same result. Since his work was not published in any foreign journals, the fact is not generally known.

This work seems to demonstrate that if transformation of sex by means of a hormone is feasible, the attempt must be made upon very young stages when little or no differentiation has occurred. Once differentiation is well begun, the evidence is that it cannot be efficiently undone. Suppression is possible and atrophic changes may occur, but it is impossible to complete upon such a foundation, the normal structures of the other sex.

This work was undertaken and carried out at the suggestion and under the direction of Prof. R. G. Harrison, to whom the greatest appreciation and thanks are offered. Special acknowledgments are also due to Dr. W. W. Swingle for advice and criticism.

Materials and preparation

The material used in this experiment consisted chiefly of the embryos of Amblystoma punctatum Linn. In preserving material, Bouin's and Zenker's fluids were chiefly used. For the smaller specimens Bouin was more often used, while most of the larger pairs were preserved in Zenker. Iron hematoxylin with eosin and Delafield's hematoxylin with the same counterstain were used for staining. For iron hematoxylin the sections were cut at 7 mm. and for Delafield's at 10 and 12 mm. In typical cases and at intervals to secure serial stages, whole pairs were sectioned. In all other cases, the gonads, together with adjacent portions of the kidney, were removed from the body and embedded in one block for sectioning.

Operative technique

The method is to join the young embryos in parabiosis at a very early stage. In grafting, various positions were tried at first: back-to-back, front-to-front, and side-to-side attachments being made. It was soon found that the last is the most satisfactory position, since it interferes least with feeding and permits more normal activity. The stage found to be most suitable for this operation comes shortly after closure of the medullary folds, when the tail-bud is well formed. In a series of embryonic stages used in this laboratory for convenience in reference, this condition corresponds roughly with stage 28. For the operation, embryos are removed from the egg membranes and placed in a small wax dish containing an 0.4 per cent solution of NaCl.

A pair of fine-pointed iridectomy scissors is used for cutting. The operation is performed under a dissecting microscope as follows: An oval area of ectoderm, covering the posterior half of the gill region back as far as the pronephric swelling is removed from the adjacent sides of two embryos, which are slipped into a pit with the cut surfaces closely apposed, and covered with a glass plate. The dish is then set in a quiet place. In from three to five hours the wound is

completely healed, and the connection is the only evidence of the operation. The mortality at the time of operation and in the period immediately following is great. This will be taken up later in the discussion of the mortality and the factors entering therein. After healing is accomplished, the glass cover is removed. The animals are still left in the salt solution for a few hours and then transferred to a glass dish containing tap-water.

History of the experiment

A number of animals were experimented upon in the spring of 1922. The mortality was high, and no exact figures as to the total number of operations or as to just when the heaviest mortality occurred are available. The series was run chiefly to ascertain the possibilities of the method. Only twelve pairs survived until metamorphosis, and none were preserved for early stages. These twelve cases constitute the 1922 series. All proved to be well differentiated at the time of metamorphosis. Of the twelve pairs of animals successfully reared in 1922, in every case both members of a pair were of the same sex, seven pairs being male and five pairs female.

In 1923, the experiment was repeated on a larger scale. Animals were killed and preserved at intervals of a few days, all through the period of larval development, in order to secure a series of developing stages. An exact record was kept of the mortality in order to check up, if possible, on the probable cause. A number of series were run, seven in all, using the eggs of several species of amphibians. The forms used were Rana sylvatica, Rana pipiens, and Amblystoma punctatum, all local species, and the axolotl (Amblystoma tigrinum), the eggs of which were laid in the laboratory. In all series except no. IV, homoplastic grafts were made; that is, joining two individuals of the same species. In series IV, however, heteroplastic grafts were tried, using the embryos of A. punctatum and the axolotl. A brief account of each series will be given, together with a table of vital statistics for that series.

Series I (Rana sylvatica) and *Series II* (Rana pipiens) need only brief mention. For some reason, not entirely determined, frogs proved unsuitable for the experiment, although Smith ('21) succeeded in rearing a few parabiotic pairs to metamorphosis. They are very readily joined, but all die, almost without exception, before ever feeding.

Series III. This series is from early-season eggs of A. punctatum sent from Baltimore, Maryland, by Dr. F. H. Swett, of the Johns Hopkins Medical School. It consists of 106 cases. A record of the series is presented in the form of a table of vital statistics, showing mortality with reference to the various periods of development and the dates on which specimens were preserved.

Certain 'critical periods' are marked on these tables for aid in classifying and interpreting mortality. The bearing of mortality, from a theoretical standpoint, will be considered later; however, the significance of these 'critical periods' may be explained here. 'Stage 36,' the period when circulation is established, is reached ordinarily about four days after operation, and those pairs which survive at this point may be considered beyond danger from immediate effects of the operation. This is the first critical period. 'Stage 46' marks the time at which feeding begins, always a critical period for 'operated animals,' and to some extent for normal animals. The handicap of being tied to another individual, and various anatomical abnormalities due to operations, puts the paired animals at a distinct disadvantage. Finally, the period beginning about July 1st marks the beginning of sex differentiation. There is no known reason for calling this a critical period from the standpoint of survival, but it is at least critical from the standpoint of the experiment. It is interesting to note how the mortality is distributed with reference to these periods.

If the table for series III be compared with the tables for the other series, it will be seen, as a rule, that when, in any series, gaps occur in the column for 'preservation,' this discrepancy is covered in other series.

The statistics appearing in table 3 show that a 30 per cent mortality occurred before stage 36 was passed. At stage 46, the approximate time when feeding begins, the mortality had reached 53 per cent. At this stage the developing sex organs are represented by the 'paired anlagen,' which are already beginning to project downward into the body cavity as the germinal ridges. By July 1st, at the time when the first appearances of sex differentiation are observable, the mortality was 71 per cent. The total mortality on February

TABLE 1

Series III

	KILLED	DIED	LIVING	
St36		32	74	Period of danger from operation
St46		24	50	Period of beginning of feeding
May 15. 12–14 mm.	8	11	31	
May 26 length variable	4	7	20	
July 1	3	2	15	Beginning of differentiation (in normals)
July 26		1		
September 30		1		
October 14	2			
October 23		1		
November 20	4			
November 22	1			
December 2	3			
January 1			2	Cases still living

5, 1924, when the last survivors were killed, was 75 per cent. In the period of seven months after differentiation had begun there was but 4 per cent mortality to be added to the 71 per cent for the entire period preceding the beginning of differentiation.

Series IV. In this series embryos of A. punctatum were grafted to embryos of the axolotl (A. tigrinum). Since no great success was anticipated with this combination, only eight pairs were grafted. The egg of the axolotl is smaller than the egg of A. punctatum, contains much less yolk, and is differently pigmented. But the most noteworthy difference

is that the young axolotls grow at a much faster rate, and arrive at the feeding stage much sooner than A. punctatum. In these pairs the axolotl, although much smaller at first, soon overtook and then exceeded in size the slower-growing A. punctatum. Of the eight pairs, three survived until June, and one lived until June 23rd. When sectioned, this pair proved to be well differentiated sexually. Both were males. The axolotl component of the pair measured 25 mm. in length and the A. punctatum but 17 mm. The interesting point here is that in all the material of the other series, nor-

TABLE 2

Series VI

	KILLED	DIED	LIVING	
St36		33	47	Period of danger from operation
St46		14	33	Period of beginning of feeding
May 28	10	6	17	
July 1		2	15	Beginning of differentiation (in normals)
July 17	1	1		
September 10	1	1		
October 14		2		
November 4	1			
November 12	2	1		
November 20	1			
November 22	2			
January 1			2	Cases still living

mal and experimental, no cases were found in which any degree of differentiation occurred before July 1st. The A. punctatum component of this pair was as well differentiated on June 23rd as were any of the animals of other series in August. This may possibly indicate that the axolotl normally differentiates earlier and influences its partner, inducing a precocious differentiation of sex. A larger series of this kind is planned for another year.

Series V. This series comprised about fifty cases in which axolotl embryos were used. The eggs were from sexually mature individuals which had been in the laboratory for some time. These seemed to survive operation better than A.

punctatum, but most of them died without feeding. None lived until time for sex differentiation. The animals developed typically an ailment in which the stomach and intestine become inflated with gas, the animals float at the surface and, being unable to feed, die. This ailment is common among young normal axolotls and in those used in other experiments.

TABLE 3

Series VII

	KILLED	DIED	LIVING	
St36		75	103	Period of danger from operation
St46		43	60	Period of beginning of feeding
May 15		11	49	
May 26		4	45	
May 28		3	42	
June 12	1	1	40	
July 1, beginning of differentiation		3	37	Beginning of differentiation (in normals)
July 26	6	1		
August 2	6			
August 28	1	3		
September 10	3			
September 30	2	2		
October 11	3			
October 20	4	1		
October 25	1			
November 4	1			
November 10	1			
December 12	1			
January 1	1			
January 5			0	Cases still living

Series VI. This series consists of eighty cases, operated between April 12th and 17th. These pairs were from eggs of A. punctatum from the vicinity of New Haven. The eggs were obtained about two weeks later than those used in series III, and fall about the middle of the spawning season. The percentage of mortality for this series and the distribution are almost exactly the same as for series III. At July 1st, the time for beginning of differentiation, fifteen pairs were

living. These were killed from time to time, the last two pairs being killed February 5, 1924.

Series VII. This series is the last and largest in point of numbers, comprising 178 cases. The operations were performed between April 20th and May 9th, and fall at the very end of the spawning season. The three principal series, III, VI, and VII, are so distributed as to cover the whole extent of the spawning season. This, together with the control series carried with each of the 'operated series,' should give a good check upon sex ratios, in case any factor, such as temperature, should in any way affect the normal proportion. In this series, also, the precaution was taken to draw the two components of a pair from different bunches of eggs, in case it should sometimes happen that the sex ratio for a single bunch of eggs is unusual. None of these precautions were justified. Both in control series and in 'operated series' the sex ratio always approximated the normal. The last two pairs of this series were killed February 5, 1924, and at that time measured 35 mm.

2. NORMAL DEVELOPMENT OF THE SEX ORGANS OF AMBLYSTOMA

Since the interpretation of the result of this experiment depends on an accurate diagnosis of sex, it has seemed necessary to go carefully into the history of normal development and differentiation of sex in Amblystoma. Hall ('04) in his work on the mesonephros and Müllerian duct in Amphibia, has touched upon this field, as have other writers, in a casual way. Spengel and Hoffman, many years ago, described the adult anatomy and the general manner of development in a number of European amphibians, but other than this little has been written on this subject.

The primordial germ cells

The problem of the origin of the primordial germ cells is not directly related to this experiment, but since the material at hand exhibits the later history of these cells, a brief ac-

count will be included here, although the early stages, which are crucial in determining the actual origin of these cells, are lacking. However, a very complete study of the whole problem of the origin of the primordial germ cells in Amphibia, and especially in Urodeles, has recently been completed by Humphrey ('24), who worked on several forms, among them Amblystoma maculata (punctatum). Through the kindness of Doctor Humphrey, the writer has been furnished with a copy of this paper, complete except for the illustrations. Humphrey has been able to follow the primordial germ cells back into stages earlier than any examined in this work, and finds that these cells, when first identifiable, are definitely in the mesoderm lying in both splanchnic and somatic layers of the lateral plate. For a full treatment of the problem of origin of the primordial germ cells of Amphibia, those interested are referred to Humphrey's paper.

The more limited material of the present study agrees very closely with the findings of Humphrey. In an embryo at about stage 36 (6.5 mm.) the lateral plate has split into the somatic and splanchnic layers, the Wolffian duct is already marked out, and beneath it, turning medialward at a sharp angle, is the median plate of the lateral mesoderm. Resting upon this plate in the angle between it and the Wolffian duct may be seen a group of primordial germ cells (fig. 1). A typical cross-section shows about three to five of these cells which are readily recognizable because of marked peculiarities. In size and in the amount of yolk contained they resemble endodermal cells, but they are clearly separated from the endoderm by the median plate. The yolk, instead of being in the form of a few huge masses as in the endodermal cells of the gut, is reduced to a number of small platelets similar in size to those in the mesoderm. These cells are so filled with yolk and a heavy brown pigment that cell outlines are not clear, although indicated by the grouping of the platelets.

From this point these cells are readily followed into the germ ridges. Humphrey has pointed out that this movement appears to be quite passive. Their location insures their

being carried toward the definitive position by the general shifting of tissues that occurs in this region. At about stage 45 (10 mm.), the two masses are practically apposed, lying on either side of the median plane, but, as Humphrey states, there is never any formation of a median anlage, as seen in Anura, where the cells arise differently. During transit there is a reduction in the quantity of yolk and an increase in the amount of brown pigment. The nuclei are also more visible as a result of the loss of yolk and are commonly very lobate or polymorphic. Lying thus in their definitive position, these cell masses have been termed the 'paired anlagen.' They rest on the median portion of the lateral plate; laterally, on either side, lie the Wolffian ducts; in the midline they are separated by a few compressed mesothelial cells that represent the anlage of the mesentery of the intestine, while above and separating them from the aorta is a small quantity of mesothelial cells comprising the nephrogenic tissue. These presently display signs of organization, coming together into groups that arrange themselves radially—the beginnings of the mesonephric tubules. This condition is seen in figure 2. With the splitting of the mesoderm and the extension of the body cavity to the midline, the 'paired anlagen' form parallel ridges, continuous above with the kidney mass and bulging below into the body cavity.

The paired germ ridges and indifferent gonad

Animals at this stage of development vary from 12 to 15 mm. The kidneys (mesonephroi) are now definitely organized into tubules and glomeruli, with a meager connective-tissue stroma between, and the Wolffian duct presents a uniformly round or oval cross-section at the inferolateral edge of the kidney mass. The mesentery of the intestine is now complete and is composed of a double fold of peritoneum. Occasionally aberrant germ cells are seen, caught between its folds, where they may persist until late stages. Such misplaced cells have never been seen to divide, but persist a while until degeneration—their eventual fate—overtakes

them. Also aberrant germ cells are not infrequently seen caught between the tubules of the kidney.

The cells of the kidney epithelium are cuboidal, and ciliated in the distal portion of the tubules. Their nuclei are small, oval, and compact. The germ cells are very much larger, with large oval, or polymorphic, vesicular nuclei that stain lightly. Here the chromatin is finely diffused except for a single densely staining nucleolus, which is usually seen. Polymorphism of the nucleus may be so exaggerated as occasionally to give the impression of two or more nuclei. The cytoplasm still contains a plentiful supply of the characteristic brown pigment.

The germ cells are now segregated into two definite ridges, along the medial edges of the kidneys, and bulging into the body cavity. The ridges may show traces of 'segmentation,' with clumps or enlargements, giving each ridge a beaded appearance. This is not always clear, however. It can scarcely be regarded as an evidence of segmental structure, nor can it be explained as due to multiplication of cells, for as yet there is no indication of mitotic activity among the primordial germ cells. These paired ridges are the germ ridges, or genital ridges (fig. 3). Topographically, they are not yet marked off from the kidney mass. This separation occurs gradually. The ridges project more and more into the cavity of the coelom, carrying with them the peritoneum as an enveloping fold. A constriction appears at either side until the germ ridges come to be suspended in a deep peritoneal fold, lying parallel to the mesentery on either side (fig. 4). Now, for the first time, other cell elements are to be seen in definite relation with the germ cells. These cells extend downward from above, following the germ cells into the peritoneal pouch. They appear in irregular, loosely organized groups or chains which are continuous above with kidney tissues, while below they are irregularly disposed among the germ cells. They are much smaller than the germ cells, being even smaller and more rounded than the average stromal cells of the kidney, though in all other respects and

in staining reaction they resemble nephrogenic cells. Through these irregular cell chains, continuity is maintained between the kidney mass above and the almost isolated gonad below, and this path is kept open by proliferation of cells from above. This is the first appearance of secondary elements that establish a definitive relation with the primordial germ cells. These cellular elements represent the anlagen of the 'sex cords.' The gonad is now definitely formed, though as yet there is no basis for differentiation of sex.

At this time the 'fat-body' first makes its appearance. It is seen in cross-section as a small bud of cells growing out medially from the hilus of the gonad. Its cells are entirely derived from the secondary elements ('sex-cord' cells) just described, and for a long time resemble these almost to identity. At first the structure is a solid bud of cells, but soon these arrange themselves radially and a small lumen appears. This lumen often persists in late stages. There is no appearance of lipoid material until sex differentiation is far advanced.

In cross-section the indifferent gonad appears as a pear-shaped pouch, consisting of the peritoneal membrane folded upon itself so that the mesentery consists of a double layer of peritoneum, separated at intervals by intruding cell groups of the interstitium. The contents of the pouch consist of germ cells—two or three to a cross-section—and mesenchymal cells that have intruded from the mesonephros. These are loosely arranged into compartments investing each germ cell (fig. 5). The germ cells still possess their characteristic large nuclei and the chromatin takes a rich hematoxylin stain. The pigment granules are greatly diminished, their color being grayish. They seem on the way to disappear entirely. Although no mitotic figures have been observed, the presence now and then of groups of germ cells of smaller size indicates that the resting period is about to give way to a period of multiplication. At a stage slightly more advanced a distinct increase in number of germ cells is seen. Nuclear polymorphism is a very conspicuous character of the germ cells. Fre-

quently it is extreme. In earlier stages, when the cytoplasm is still filled with yolk, it might easily be assumed that the condition is a deformation due to pressure of the platelets, but the condition persists and is seen in all subsequent stages until differentiation begins. At this time the condition disappears in young oocytes, which come to possess round or oval nuclei, but it is still a common characteristic of the spermatogonia, being developed to such an extreme that the appearance of the nucleus is often that of an aggregation of irregular confluent vesicles.

In animals 18 to 20 mm. in length (ten to twelve weeks in age) the gonad has increased considerably in size. Mitotic figures appear and the number of germ cells to be seen in a single cross-section varies from five to seven. Chromosome counts were attempted, to see whether sex could be determined in this way, but the number of chromosomes is large and the figures so closely clumped that the hope was given up. According to Parmenter ('19), who worked upon the chromosomes of Amblystoma tigrinum, a sex chromosome is not certainly recognizable, but is possibly attached to one of the autosomes.

There is still no noticeable change in the relation of the elements and the gonad is still indifferent. The germ cells are casually distributed among the secondary cells, tending in the main to lie toward the periphery, while the center of the gonad is occupied by a core of secondary cells. Most of the cases preserved between June 15th and July 1st are in this group. Age, however, is a more reliable index for time of differentiation than is size. Due to differences in ability to feed readily or to unfavorable factors in the laboratory environment, there is often considerable discrepancy in size between animals in the same series. This is true among normal animals.

Hall ('04) finds for the laboratory environment the same unfavorable effect upon normal animals, even when tended as carefully as possible. He also states that the size of the individual, within considerable limits, has little significance as an index for degree of development.

Differentiation—female

Differentiation of sex is first noticeable at about ten or twelve weeks of age, and taking an average for the different egg cultures this period falls about July 1st. Since secondary sex characters are practically lacking even in adult individuals except for a brief time during breeding season, sex can be determined only by sectioning the gonad. Differentiation has to do chiefly with morphological changes brought about through rearrangement of elements within the gonad accompanied by a conspicuous change in the cell nucleus. In the female gonad the germ cells take a peripheral position where they form a uniform 'germinal layer,' so that in cross-section the ovary appears as a hollow sac (fig. 9). Another factor, which in itself may be considered an important criterion for sex, is the cessation of growth in the 'sex cords.' Proliferation of secondary cells from the mesonephros into the young ovary stops rather suddenly, so that the cavity which is formed at this time is not obliterated. These changes occur as follows:

The germ cells, now to be called the primitive oogonia, form a single layer around the periphery of the gonad, with a few interstitial cells interposed in an irregular layer, between the oogonia and the peritoneum. The secondary cells, which in the undifferentiated gonad form a central core, spread peripherally, leaving a lumen or ovarian cavity. The very young ovary, then, is a simple sac, covered without by the peritoneal membrane reinforced by a few mesothelial cells and lined within by a thin mesothelium. Between these layers lie the primitive oogonia, forming a single rank, partially isolated from each other by a scanty interstitium.

The primitive oogonia are very little larger than the primordial germ cells. Individually, they are recognizable as oogonia solely by the character of the nucleus, the cytoplasm being relatively small in amount. In the nucleus, however, there is a conspicuous change in the organization of the chromatin. Spireme-like 'pseudo-maturation figures,' are

characteristic of all but the youngest oogonia (figs. 9 and 10), which apparently continue to divide for some time. A nucleolus is usually present, sometimes more than one. Yolk begins to appear in the form of granules which increase rapidly in number with the growth of the cell. All these phenomena are correlated with the growth period upon which the oogonia are just entering. Increase in the size of the oogonia causes them to be shunted inward toward the ovarian cavity, into which, as their size continues to increase, they begin to protrude (fig. 11), and the extent of the cavity is greatly decreased in consequence. All the younger oogonia remain at the periphery, still maintaining a more or less uniform and continuous germinal layer (figs. 10 and 12). Occasionally a single rapidly growing oocyte will expand and completely fill the ovarian cavity, lying there ringed about by its smaller sister cells.

As growth progresses and deposition of yolk increases, the cells assume more and more the character of typical oocytes. Correlated with this there is a gradual regressive change in the nuclear spiremes, which become more and more indistinct, but for some time maintain a coarse-meshed reticular pattern that suggests a shadowy spireme.

The later development of the ovary shows no important morphological changes. This period is concerned mainly in a rapid and enormous increase in the size of those oogonia already entered into the growth period (figs. 11 and 12). The young germ cells of the germinal layer are still confined entirely to the periphery of the gonad, where they form a layer of smaller cells, poor in cytoplasm, but with large nuclei that show reticula are even spiremes. The ovarian cavity is now occluded and finally completely obliterated, and as more and more of the young oogonia enter into the growth period and the rate of multiplication falls off there is a depletion of the germinal layer until it has practically disappeared as a defined layer. Finally, as metamorphosis approaches, the gonad is a solid structure, packed with large, fully grown oocytes. Young oogonia at this stage are lacking entirely in

some sections; however, other sections may show several, and even nests of young cells are seen. Their position, whether as single cells or as cell nests, is always peripheral, with a tendency to locate near the hilus of the gland (fig. 12).

The interstitium of the ovary is rather scant at all stages. In the later stages this is the more noticeable because of the sudden enlargement of the gland due to growth of the oocytes. While there is probably some absolute increase in the number of interstitial cells, it does not by any means keep pace with the increase in volume of the gland as a whole; consequently, the follicles of the oocytes scarcely justify the name. In cross-section, a single large oocyte is seen to be surrounded by a thin ring of greatly flattened cells which are stretched to an abnormal thinness. The nuclei of these follicle cells are round or oval in plane dimension, but, due to stress, are very greatly flattened. Just beneath the peritoneum an ill-defined single layer of similar cells seems to reinforce that membrane. This layer is occasionally thickened to form a nest for a few young oogonia, and at the hilus of the gland a wedge-shaped aggregation of interstitial cells is usually found. There is nothing definite in the character of these cells to indicate that they possess a capacity for internal secretion. In sexually mature adults a secretory capacity may be developed, though Humphrey's findings in other Urodeles (Humphrey, '21, Interstitial cells in Urodeles) do not favor this. However, the interstitum will be reconsidered later in connection with the question of the sex hormone.

Differentiation—male

For a time after the prospective ovary is distinguishable as such, there is difficulty in distinguishing the young testis from the indifferent gonad. Both structures are of the solid type, no indication of a lumen appearing (fig. 6). A gland in question may represent a young stage of testis development or it may merely be still indifferent, with the possibility of developing later into the female type. This is the

only point in development where cases of doubtful interpretation are encountered. The period is not of long duration, so it is not probable that the sex ratio has been affected to any extent by doubtful cases wrongly interpreted. When in doubt it has been the practice to consider the case as undifferentiated. The possible effect of such classification upon sex ratios will be considered later. The total number of such cases is not great, however.

The change in structure that at once distinguishes the differentiating testis from the indifferent gonad is exactly opposed to that which occurs in the young ovary. The spermatogonia scatter themselves evenly through the interstitium. This gives to the testis a compactness and homogeneity of structure that is quite in contrast to the hollow, saccular ovary. Also the spermatogonia remain small, very little larger than the germ cells of the indifferent stage, which they resemble in other ways than in size. There is nothing even faintly suggesting the nuclear reticula and spiremes of the young oocytes. It seems that the spermatogonia for a time maintain the same division rate that was theirs as undetermined germ cells in the indifferent gonad. The first factor, then, in the growth of the testis is the prolongation of the multiplication period of the germ cells into the first stages of differentiation.

The second factor is one which is properly its own. The proliferation of the 'sex cords,' which is checked in the ovary at the very beginning of differentiation, continues in the testis. The sex cord material is invaded and broken up by the spermatogonia until the whole mass is thoroughly dispersed throughout the gland except for a small entering wedge at the hilus. This dispersal of the sex-cord material gives to the testis the uniform structure that is its most apparent characteristic. There is no nesting of the spermatogonia in early stages of differentiation. The individual cells are scattered singly through the interstitium.

Because of the continuous proliferation of the sex cords, together with the failure of the testis to expand to anything

approaching the proportions of the ovary, the interstitium of the testis is relatively much more abundant (fig. 7). Also, due to the fact that it is not subjected to such tension, the cells are more rounded in contour than those of the ovary.

The later history of the differentiation of the testis shows but one process that is noteworthy. This is the further growth and consolidation of the sex cords, and in conjunction with this process the nesting of the spermatogonia in groups, which are the anlagen of the lobules of the mature testis. (There is an additional increase in size, but this is not great, and at no time does the organ even approach the ovary in size.) It is probable that all the inmates of a given nest are descendants of a single cell. The number is never great, four being most commonly seen; sometimes only two occur, and occasionally a considerable number are grouped. Mitotic figures are still found, bearing out this conclusion. Between the germ-cell nests the interstitium forms thin but very compact lamellae, which cut up the interior of the gland into several compartments, varying somewhat in size. In a typical cross-section of the testis at this stage there will be seven to eight such compartments, arranged bilaterally with reference to a plane passed through the long axis of the cross-section. This plane contains the entering wedge of sex-cord material. A testis in such a stage of development is seen in figure 8. The axial core of interstitium in the mature testis gives rise to the system of collecting ducts, or rete, while in the thicker portion, near the hilus, a longitudinal collecting duct arises, by the appearance of a lumen in the cord. The lamellae going off from the axial cord, as partitions between the nests of spermatogonia, will in a similar way form the lobular ducts; while each nest, by multiplication of its cells, forms a definitive lobule of the mature testis.

A testis in the condition just described is fully differentiated, although not mature, since there are no ripe sex products formed. Humphrey ('22), in his study of the multiple testis in Urodeles, shows that after the sex products are ripened and discharged, the lobule goes into a regressive

phase in which almost all the germ cells completely disappear. At this time there is a considerable increase in the relative amount of interstitium. All lobules in this regressive state appear completely degenerate, even the lobular duct returns to a solid cell-cord stage. However, in preparation for the next breeding season, the germ cells are regenerated from 'residual spermatogonia' which survive the process of degeneration, and by multiplication of these, cell nests are formed, and finally the lobule is completely restored. This regressive phase of the lobule seems, then, to lead back to a condition resembling that seen in immature males at the very end of larval life.

Comparison and contrast of development of ovary and testis

1. In the period of development described as the 'indifferent gonad,' there are no means by which we can distinguish sex in the larvae of Amblystoma.

2. With the beginning of differentiation an abrupt divergence in type occurs. In the female gonad there is a movement of the germ cells to the periphery, where they form a definite germinal layer, together with the development of a wide ovarian cavity through the longitudinal axis of the gland. In the male a converse process takes place. The germ cells distribute themselves uniformly through the substance of the gland, producing a gonad of solid, homogeneous structure.

3. The next step in the developing ovary is the entrance of the older oogonia into a growth period, accompanied, first, by a change in nuclear organization, producing the 'false-maturation figures'—chromatin reticula and spiremes. This is followed by great increase in the amount of cytoplasm, with the formation of yolk. The cells become typical oocytes, though maximum size is not yet entirely attained. In the developing testis no such phenomena are ever seen. The young spermatogonia undergo slight change. There is little increase in size and the similarity to the germ cells of the indifferent gonad is close. There is no change in nuclear or-

ganization and the period of multiplication seems to be extended.

4. In the young ovary the proliferation of sex-cord material from the mesonephros ceases. This permits the ovarian cavity to exist as such. In the male proliferation of secondary cells continues until metamorphosis or perhaps later, and the definitive tubules are marked out. Nesting of the spermatogonia occurs. The gland remains small in comparison with the bulk of an ovary at the corresponding stage.

5. The amount of interstitium in the ovary seems rather small in comparison with the testis. There are two reasons for this condition. In the ovary the great expansion of the gland through growth of the oocytes leads to a disproportionate increase in bulk of the sexual elements relative to secondary elements. The second factor is the continued proliferation of sex-cord material in the testis.

Most of the characters here adopted as criteria have been previously described by writers on sex differentiation in amphibians, or, if not specifically described, have been figured by almost all who have written upon the question.

3. CONDITIONS FOUND IN OPERATED ANIMALS

Specimens were preserved at intervals of a few days, all through the period of larval life, and at the present time (February 1, 1924) four pairs of animals joined in April, 1923, are still living, two pairs belonging to series III are about 35 mm. in length, while the two remaining pairs, belonging to series VII, are about 25 mm. in length. In early stages sectioning is necessary to determine sex, but at metamorphosis, or shortly before, sex can readily be determined by gross dissection. Müllerian ducts are present and equally developed in both sexes, but the gonads differ greatly in size and appearance (pl. 6). The testis is small, slender, spindle-shaped, and of a homogeneous texture. In contrast, the ovary is vastly larger, lobate, and, because of the follicular structure, has a very characteristic texture. The follicles are visible to the naked eye as dull white spherules, embedded in a more transparent stroma.

It is noteworthy that in all save series IV, where different species were grafted, growth was remarkably uniform throughout life. In fully 90 per cent of all cases, even when of some size, no difference in length could be detected except by careful measurement. In some cases there was a difference in girth, correlated with asymmetry of internal organs in the region of fusion; or more often with the fact that one member of the pair did most of the feeding. Rarely, one member of a pair was never observed to feed, yet at metamorphosis was, in length, the equal of its companion. Exceptions to this uniformity of growth, not more than six in all, were found only in cases where injury from operation proved deforming.

Anatomical modifications

The most important anatomical modification, from the standpoint of the success of the experiment, involves the circulatory system. Working on the principle of a hormone carried in the blood stream, an essential point is the existence of an adequate vascular connection between members of a pair. This condition is well fulfilled. All paired animals are closely joined from the gills to a point a short distance behind the pectoral girdle, therefore including the entire region of the heart and the area of the branchial circulation. This resulted in a connection so close that all degrees are found between very considerable connections of the chambers of the heart to almost complete fusion into a single organ. In addition to this are found important connections of smaller vessels, such as branchial vessels. Obviously, this means a single blood stream for the two individuals and settles the point satisfactorily.

The second organ system to be greatly affected as a result of the experiment is the alimentary tract. The degree of union found here is more variable and important indirectly from its relation to well-being and survival. Various degrees of fusion were found from the oesophagus as far back as the duodenum. Common types of connection between the alimen-

tary tracts are: a common oesophagus, common stomach, common duodenum, lesser communications in any of these regions, and, in addition, almost any combination of these connections. There are many cases in which the canal, posterior to the last important cross-connection, remained functionless in one member; both members feeding well, but with all elimination performed by one. Often the non-functional canal became partially atrophied, occlusion of the lumen occurring. There are a few cases in which one member of the pair never fed, living entirely in the manner of a parasite upon its partner, which also demonstrates the degree of community existing between the blood streams, since almost invariably such a parasitic individual grew as well as the host, maintaining an equal length at all times, although the girth always remained markedly less, due to the fact that the organs of digestion were collapsed. That such intimate connection occurs as a result of the operation is unfortunate, but apparently unavoidable. Numerous constrictions occur in the regions of connection, leading to functional disturbances and complete clogging of the canal, resulting in death. A large part of the mortality is directly traceable to this source.

Another way in which the joining of members of a pair works to their disadvantage is in the feeding act. The young larva feeds upon small, actively moving water organisms, and so, if capture is to be successfully accomplished in a sufficient number of cases, quickness of movement and accuracy of coordination are necessary. It is clear, and observation bears out the assumption, that movement is both deflected and greatly hindered by the inert mass of a non-cooperating partner, and that the union in the region of the branchial chamber interferes with the normal action of the jaws and pharynx. This handicap easily accounts for the unfavorable comparison, as to growth and well-being, between animals of the operated series and normal individuals, and is a prime factor in mortality.

Structure of the gonad in operated animals

Although this part of the investigation is necessarily of great importance, very little space is needed, because of the unexpected simplicity of the principal findings.

Careful examination of the material failed to show a condition of intersexuality in a single case or any histological or cytological indication that a transformation of sex had occurred. Members of a pair were invariably of the same sex and at about the same stage of differentiation. True, a considerable difference in size frequently exists between the gonads of members of a pair, but such differences are very common among normal animals of the same age and size, reared under the same conditions. Indeed, variation rather than uniformity seems to be the rule where size of the gonad is concerned. Not only is there variation in normal individuals of the same age and length, but bilateral inequality is common. This condition of bilateral inequality is also met with in the animals of the operated series. Size of the gonad, then, is no evidence for or against the possibility of sex transformation. It was for this reason that a study of the developmental stages and differentiation of sex in normal animals was undertaken, in order to determine what criteria exist that are reliable for distinguishing sex in the early stages of differentiation. This part has already been presented, and it seems to establish the fact that there are two readily distinguishable types of gonad, diverging at an early period. It is not easy to be mistaken in type, except perhaps for a short period at the very onset of differentiation. Comparison of the gonads of the operated series with types of the normal series leads to the conclusion that the development of the gonads of the animals of the experiment is, in all observable respects, *normal from the beginning of differentiation.* What may have occurred before that time is problematical and will be deferred, together with theoretical considerations, for later discussion. The complete description of differentiation in normal forms is considered to apply equally to animals of the 'operated series.'

Sex ratios in operated animals

The normal sex ratio occurring in Amblystoma is 1:1. This ratio is closely approximated in animals brought in from the natural habitat and in groups raised for control purposes in the laboratory.

The twelve pairs of animals raised in 1922 showed upon examination 5 ♀ ♀ and 7 ♂ ♂. These animals were all kept until the period of metamorphosis. The number of pairs in 1923 was much greater. The total number of cases in which sex was positively identified (most of them by sectioning and histological study) is 80. Many others examined were still in the indifferent stage and could not be classified. The total number of cases sectioned is about 100. The 80 pairs positively identified show 36 ♀ ♀ and 44 ♂ ♂. It is seen that the numerical advantage lies with the males, but this advantage is scarcely great enough to seriously disturb the normal 1:1 ratio. But liability of error (which is hardly very large in any case) would affect the number of males. It will be remembered from the description of the early stages that for a very brief space there is some difficulty in distinguishing the male type from the 'indifferent gonad.' Since this period is relatively short, the number of cases falling therein is not great. If identification was not fairly certain, as happened in a few cases, the practice was to consider the case as undifferentiated. There is no such questionable stage in the differentiation of the female gonad. It is apparent that any error here is not likely to disturb seriously the ratio as given above and might tend to cause a closer approximation of the 1:1 ratio, if, for example, a case classed as young male should prove to be merely undifferentiated. How this compares with the expected ratio, and the significance and explanation of this ratio as it stands, will be considered again in the final discussion.

Mortality

The mortality in all series was high. Series I, II, and V (frogs and axolotls) were completely destroyed, and series

IV, a small series to begin with, had but one pair which survived until sex was clearly differentiated. These series are omitted from further consideration. A high mortality was encountered in the small number of cases tried in 1922, and in 1923 an effort was made to reduce the percentage, but without success. The total mortality for all the series considered is nearly 78 per cent, and the series, when considered separately, fall very near this average. Table 4 shows the exact percentage of mortality in each series, grouped according to 'critical periods' in development, for the purpose of direct comparison. For the significance of these 'critical periods,' see page 68. Without doubt there are many reasons for this high mortality, most of which is undoubtedly to be expected under the conditions of the experiment. Among these may be mentioned: injury incident to operation, malformation and handicap resulting from operation, unsuitable laboratory environment, etc.

4. DISCUSSION AND CONCLUSIONS

The determination of sex

The question of the determination of sex and the factors involved therein is far from being solved in a satisfactory way. There is a considerable mass of evidence that is contradictory to the view that sex is definitely determined at the time of fertilization. It is probably true that in the normal course of development sex is thus determined in a vast majority of cases, and the body of evidence, cytological and experimental, that has been built up around the sex chromosome as the mechanism of sex determination cannot be gainsaid. But whether this view can be given a literal and unqualified acceptance would seem to depend upon the nature of those hereditary characters that determine sex, and upon the manner in which they eventually operate.

The 'fertilization theory' does not formulate the determining factors in physical terms nor provide a mechanism for their operation. The theory of Goldschmidt is more specific in these respects. It furnishes a definite physiological basis

for the problem. Goldschmidt accepts the main idea of the 'fertilization theory'—that the sex-determining factors are distributed through the agency of the sex-chromosome mechanism—modifying it to the extent that there are two opposing sets of factors, one of which (that for femaleness in Lymantria) is carried elsewhere, probably in the cytoplasm of the egg or in a Y-chromosome. He considers factors for maleness and for femaleness to be present in every fertilized egg in a definitely quantitative relation. The given sex is the resultant of the proportionate relation existing between these quantitative determiners. If we assume—and Goldschmidt does so—that these factors, having a definite quantitative value, are distributed to the fertilized egg by the sex-chromosome mechanism, then we have these two theories in accord—in fact, supplementing one another. The recent work of Bridges ('23) on Drosophila, moreover, seems to indicate clearly that sex is a resultant of the interaction of two sets of factors, probably of a quantitative nature, one of which is carried on the sex chromosome and the other on the group of autosomes as a whole. This falls in with Goldschmidt's hypothesis, except that it assigns one set of factors to the group of autosomes instead of to the cytoplasm; a more satisfactory arrangement in view of the definitely hereditary character of the transmission of sex. To go a step further, the 'quantitative determiners' of Goldschmidt, as he himself proposes, are readily conceivable as determiners for hormones, or as hormone-forming substances, which at once establishes an essential cooperation with the last of the three most acceptable theories of sex now extant—the 'hormone theory.' This theory does not encroach upon the ground covered by the two preceding theories, but is really a 'theory of sex differentiation,' and rests upon a proved physiological basis.

According to the hormone theory, differentiation of sex takes place under the influence of sex hormones, formed within the young gonad, probably by the interstitial tissue, and carried by the blood stream to all parts of the body. The evidence in support of the 'hormone theory' will not be taken

up here, since the present objective is the correlation of existing theories of sex determination into a unified workable hypothesis, into which the facts of heredity, cytology, and physiology are readily adaptable. Each of the theories mentioned is well supported by cytological and experimental evidence, and it would seem that there are no serious contradictions to be overcome.

We have before us to consider, then, hereditary factors that determine sex, which factors are distributed to the zygote by the well-known sex-chromosome mechanism. These factors, or determiners, are to be considered as possessing definite quantitative values, or even as definite quantities of physiological substances, which in the course of development are translated into amounts of hormone having the same quantitative relation. This hormone complex then becomes the active physiological principle in the process of sex differentiation. In the light of such a reasonable and practical hypothesis, sex is dependent not upon an unalterable determination at the time of fertilization, but upon the normal fulfillment of a progressive physiological process, initiated by the determining factors introduced into the zygote at fertilization. Clearly, any influence which will affect the normal production of a given hormone, either to increase or to diminish it relatively, or any influence affecting the activity or potency of a given hormone, may be expected to produce an end-result analogous to that which would follow an actual alteration in the complex of hereditary factors. From the standpoint of this experiment, it may well be considered that the introduction of sex hormones—considered as end-products of sex-determining factors—from the body of one animal into another is equivalent in effect to the actual presence of such determining factors in the original hereditary complex of the animal. Both of these means, namely, variation of hereditary factors (Goldschmidt, Bridges) and manipulation of hormones, have been used experimentally to modify sex. All of the enormous amount of work done on castration, transplantation of gonads, injection of hormones (extracts from the gonad), etc., comes under the method last named.

Intersexual conditions—reversal of sex

The degree of modification ('intersexuality'—so-called by Goldschmidt and others) which can be produced depends, in general, upon the extent to which the hormone supply can be controlled experimentally and upon a time factor, or 'timing mechanism' which regulates the time, with reference to the differentiation of the organs, when the experimental factors become effective. Goldschmidt developed this idea to explain the progressive modification of organs seen in certain series of intersexes. In general, we may say that the extent to which an organ is modified depends upon how early in its development it becomes subject to the influence of the hormones introduced by the experiment and upon the duration of the influence. Any organ or system of organs whose entire developmental history is passed under the influence of such hormones may show complete modification or transformation. If the overriding influence be present before there is any morphological differentiation of sex, sex reversal may be expected. Goldschmidt so explains his cases of complete sex reversal, and the evidence in support of the explanation is clear. Such an explanation, however, demands that a 'dominance' or prepotency for one hormone be established at some point in development. The establishment of a prepotency is aided by the demonstrated independence of action possessed by hormones in general, often resulting in that type of physiological response known as the 'all or none' response. This type of response is well known in many physiological phenomena. Goldschmidt postulates such a reaction; Lillie has advanced the same theory in interpreting the intersexual conditions found in the 'free-martin,' and Pézard also has recently subscribed to the same view. When such an 'all or none' response has occurred and prepotency becomes effective before there has been any morphological differentiation, we may expect to find a complete reversal of sex. In the free-martin differentiation of sex is usually well begun before the anastomosis of foetal vessels is

accomplished, hence only an intersexual condition results, so far as known. A case of complete reversal, of course, would be unnoticed probably, but, as Lillie has recently pointed out, a study of sex ratios indicates that complete reversal does not occur.

Isolated cases of sex reversal not experimentally induced have been reported from time to time in various animals. Crewe ('23) reported a series of fowls showing all degrees of intersexuality culminating in complete reversal of sex, even to normal function. A hen that had previously laid eggs from which normal chicks were hatched, later was transformed into a cock and became the 'father' of other offspring. Riddle has recently reported a case in the ring-dove and, moreover, believes (Riddle, '16) that in pigeons sex is a potentially reversible phenomenon. Crewe ('21) also described varying degrees of sex reversal in frogs and toads, including at least one case of complete functional reversal. Another recent case is that of the teleost fish, Xiphophorus, studied by Essenberg ('23). Complete functional reversal of sex also occurred here, and Essenberg seems to show that this is a normal occurrence in a certain percentage of young developing females. Champy ('21) reports the transformation of a male Triton into a female, following prolonged starvation, and consequent degeneration of the testis. Finally, there is the well-known but disputed view of Hertwig, Kuschakewitsch, and Witschi, that reversal of sex is the usual thing in the larval life of certain races of frogs. The fact seems established that a complete reversal of sex is possible on sound theoretical grounds, and moreover does actually occur in some animals. It may well be that most cases of 'hermaphroditism' reported in various animals from time to time are not true hermaphroditism, but a condition of intersexuality induced by a derangement of the normal embryonic physiological mechanism, whereby the sex hormones are prevented from playing their normal rôle.

Experiments on castration and transplantation do not prove or disprove the possibility of reversing sex. Unfortunately,

almost all work along this line has been of necessity done on adult or well-differentiated forms, where structure is too well established to be more than slightly modified by such experiments. Modification of secondary sex characters, behavior, instincts, etc., has been obtained, but no considerable effect upon the more fundamental organs of sex. The work of Steinach, Moore ('19), Lipschütz ('18), Bresca ('10), and many others has demonstrated this. But experimental work so devised that influence is brought to bear during the period of sex differentiation has shown that in this formative period, when structure is in a more plastic state, considerable modification may occur, through manipulation of the production or the distribution of hormones. Minoura ('21) succeeded in modifying to a considerable extent the sexual parts of chick embryos by grafting pieces of testis to the allantoic membrane.

Parabiosis as a method has been used before in an attempt to modify sex, but since only adult or well-differentiated subjects were used, only mild modifications were induced. The work done is cited in part 1 of this paper.

The interstitial cells of amphibians

In amphibia there is strong evidence that the gonad has an internal secretory function as in higher vertebrates, although typical 'interstitial' cells are not developed to the same extent as in birds and mammals, either as to type of cell or as to amount of the interstitial tissue. It seems well established that secondary sex characters in Amphibia are dependent upon the presence of the gonad. Some work has been contradictory on this point, but most of such work consists of castration and transplantation experiments done upon forms where the secondary sex characters are inconspicuous and variable, and therefore not favorable for such a study. The work of Nussbaum ('09), Meisenheimer ('11), Smith ('13), and others indicates that in anurans the thumb-pad and the psychic reactions of sex are entirely dependent upon the presence of the gonads, and the work of Bresca ('10)

on Triton cristatus, where the secondary sex characters happen to be very conspicuous, also shows this very strikingly. Bresca obtained almost complete reduction of the very conspicuous dorsal crest of the male Triton following castration and also the disappearance of equally characteristic color markings. What is more significant, he found that when a strip of skin from the dorsal midline of a female is transplanted to a male in place of his own crest, the transplanted tissue develops into a typical crest. Also strips of skin from a male, and bearing the characteristic white tail stripes, lose these markings when transplanted to a female, but retain them when transplanted to another male. All of this is the best of evidence for the presence of a sex hormone in Amphibia that is quite comparable to that of birds and mammals and permits us to feel secure in the assumption of sex hormones in Amphibia that operate in a typical manner. There has been no indication in any of this work of a transformation of sex as regards primary structure, but the same criticism before advanced, holds again—that all such experiments have been made upon adult forms.

The stromal cells of the gonad of Amblystoma are not remarkable in any way. They resemble the ordinary mesothelial cells of the kidney with which they have a common origin. As described in another part of this paper (see history of normal sex differentiation), they are derived from the nephrogenic mesothelial cells of the kidney mass, follow the primordial germ cells into the 'germ-ridges,' and so are present in small numbers from the very beginning of the history of the gonad. The original number is augmented, as development goes on, partly by multiplication, but chiefly by proliferation of cells ('sex-cord' material) from without. This proliferation appears to be checked much earlier for the ovary than for the testis, which probably accounts for the relatively more abundant interstitium of the testis, although in the ovary the great expansion, due to growth of the oocytes, certainly produces a wider dispersion of the interstitium, which in part creates this impression.

The amount of interstitium is small in comparison with that of the gonad of higher vertebrates. There is no apparent differentiation of a secretory type of cell in either sex, the only obvious function being a mechanical one. The cells are small in comparison with the germ cells, the nuclei are compact and stain densely. There is not much increase in size except in late ovarial stages where a considerable increase is noticeable, accompanied by great flattening and close application to the surface of the oocytes. Champy ('08, '12, '13) in different anurans describes interstitial cells which are characterized by granules, lipoids, etc., and which resemble typical cells of internal secretion. The period of greatest abundance of these cells is inversely correlated with the cycle of spermatogenesis. Other writers describe this correlation as being direct; however, the greater weight of evidence, particularly if higher vertebrates are considered, is in accord with Champy's findings. A recent extensive study of such cells, in urodeles, from a morphophysiological standpoint, has been made by Humphrey ('21) in his paper, "The interstitial cells of the urodele testis." He considers the 'interstitial cells' of Champy and others to be but a temporary modification of mesothelial, or stromal cells, which round out, increase somewhat in size, and accumulate lipoids during the regressive phase of spermatogenesis. No specific secretion can be demonstrated, and these cells are not typically present in immature individuals. The status of these cells as an organ of internal secretion is doubtful. The findings of the present experiment agree that no cells of a typically secretory nature appear in immature individuals, although no specific cytological technique was applied. The chief argument for the existence of an organ of internal secretion for sex lies in the experimental evidence cited, and it is possible that such a hormone is the product of the germ cells themselves.

Analysis of probable causes of mortality

We have now to consider what is probably the most potential criticism of the interpretation given for the present to the results of this experiment. This is, the possibility that a 'selective' mortality has operated to eliminate all heterogeneous (male-female) pairs, permitting only homogeneous pairs to survive. With a total mortality of 77 per cent, it is impossible to refute this criticism entirely, so it must remain as a possible explanation, although it finds no support in the extensive work on transplantation. Throughout the literature of experimental transplantation and grafting of tissues there is no clear evidence of an 'antagonism' that is specific for sex. A great deal of such experimenting has been carried out, and all that has ever been shown is that the sex hormones exercise a differential influence upon the growth and differentiation of the gonad and the secondary sex characters. 'Differential' and 'antagonistic' are not equivalent terms. Moore ('21) makes such a distinction, pointing out that the first effect requires a positive stimulus, while the second implies an inhibition or negative action. His experiments on rats, in which a reciprocal transplantation of one gonad was made, bear this out. On ovary placed in a male rat possessing one functional testis grew there in a normal manner, the Graafian follicles growing to full size, though never undergoing maturation. This condition was found after the end of eight months. The reciprocal transfer behaved similarly except that the spermatogonia practically all disappeared; however, this result invariably follows any transplantation of the testis, even autoplastic grafts. Sand ('19) has actually inserted grafts of ovarian tissue into the center of a testis and finds that they grow there with no indication of active antagonism. Cases of true hermaphroditism, functionally normal, have been not infrequently reported in Amphibia, as an example of which may be cited the very clear case described by La Valette St. George ('95). An hermaphroditic Triton showed well-developed testes containing ripe spermatozoa and, beside

them, equally well-developed ovaries with typical oocytes. The secondary sex characters of the animal were those of a male. No very marked antagonism could have existed in this case.

But the case that seems to apply best to the present experiment is the work of Bresca ('10), who experimented upon the secondary sex characters of the Urodele, Triton, where sexual dimorphism is great. Large areas of skin from the dorsal midline of the female were transplanted to the corresponding position on the male, and vice versa. Also large areas of skin from the lateral surface of the tail fin, and which bear the conspicuous color markings that are a secondary sex character of the male, were transplanted to the female. The results of these experiments, from the standpoint of the regulation of secondary sex characters, have been considered elsewhere. The point to be emphasized here is that in these transplantations, involving interchange of large areas of tissue between individuals of different sex, not only were the grafts tolerated in their new position, but they 'took' so well that marked differentiation of the transplanted tissues occurred; witness, the development of the great 'dorsal crest' of the male Triton from female tissues. An antagonism so profound as to induce fatal complications in the 'two-sexed individuals' must presuppose a physiological incompatibility so fundamental as to prevent any toleration of tissues from the other sex, which is manifestly not true.

The action of sex hormones affects primarily the sex characters, primary and secondary, and abnormality of the sex organs that are not pathological are not known to be dangerous; moreover, 'intersexes,' wherever found, are not usually less viable than normal individuals, except when the condition is the partial expression of serious and more extensive hereditary complication. It does not appear that the mortality in the 'free-martin' is greater than in ordinary twins; indeed, the female twin, which is the member affected, is of a sturdier type than the normal female. While the possibility of a 'selective' mortality may exist then, there seems to be little in its support.

On the other hand, is it necessary to look beyond ordinary causes to account for the mortality that has occurred? Certainly not. A variety of causes may be cited, but none of them can be considered unusual under the conditions. One source of loss, purely general and incidental, is the weeding out of individuals that are on the whole less resistant and viable. A certain mortality may always be expected, even in cases of minor operations. But the principal causes of mortality are the effects of a laboratory environment that is distinctly unfavorable and the effects of the operation, direct and indirect. This last factor (or complex of factors) has been described in detail in part 3, where it is shown that,

TABLE 4

Mortality compared in series III, VI, and VII. Figures given are percentages of the whole number of cases in each series

	STAGE 36 PERIOD OF OPERATION	STAGE 46 BEGINNING OF FEEDING	JULY 1 BEGINNING OF DIFFERENTIATION	TOTAL MORTALITY
Series III	30	53	71+	75
Series VI	41	59	69	75
Series VII	42	66+	78	83—
Average	37+	59+	73—	77+

in addition to a serious initial injury at time of operation, the paired animals are subjected to a permanent handicap, malformation of vital organs always ensues and their activity is greatly hindered (p. 55). Consequent functional disorders are frequently the direct cause of death. That the mortality is high is not surprising.

Furthermore, a rather important fact appears when we consider the time of occurrence of mortality with reference to those periods of development that have been described as 'critical periods' (p. 37) for evident reasons. It is interesting to see that the heaviest mortality appears around these periods and that there is marked uniformity, in respect to this, in all series. Finally, *80 per cent of all deaths have oc-*

curred before July 1st, the time when differentiation of sex begins. A point to be considered, however, is that the mortality is involved with the statistics for killing, because of the practice of choosing for preservation those cases which seemed least likely to survive. It seems impracticable to unravel the two sets of figures, and the extent to which the mortality statistics would be affected is problematical.

In conclusion, we may say that, although the mortality is very high, yet it may be readily accounted for without recourse to unusual causes. This, plus the manner in which the mortality is grouped around certain periods in the life-history which may properly be considered 'critical,' and the fact that the majority of deaths occur before differentiation of sex begins, is considered significant.

An interpretation of the results of the present experiment

The foregoing considerations afford an acceptable basis for an interpretation of the present experiment, although the interpretation now offered is not to be considered as final until further work has been done. But before an explanation is attempted let us restate as concisely as possible the results obtained.

Of the eighty pairs of animals that survived until sex could be positively identified, forty-four pairs were ♂ ♂ and thirty-six pairs were ♀ ♀. No ♂ ♀ pairs appeared. The ratio of the sexes approaches the 1:1 ratio found in normal animals. Histological examination of the gonads revealed no intermediate or modified condition, and identification of sex seems positive, since the conditions found in urodeles are simple, distinct, and offer little possibility of confusion. The mortality was high, being 77 per cent, but by far the greater part of this mortality (about 80 per cent of all deaths) occurred before the period of differentiation of sex and seems readily accountable on ordinary grounds, such as effects of operation, handicap, and laboratory environment.

Unless a 'selective mortality' has been operative, the results indicate a transformation of sex, since not a single ♂ ♀

pair occurred. Yet originally many such pairings must have been made. In making random combinations of eggs the theoretical expectation as regards combinations of sex, would be 1 ♂ ♂ : 2 ♂ ♀ : 1 ♀ ♀, since, in bunches of eggs, those determined for male and for female occur in equal numbers. For example, out of forty-two larvae, past the point of differentiation, which were brought into the laboratory for examination, twenty were ♂ and twenty-two ♀, and controls reared in the laboratory showed the same close approach to a 1 : 1 ratio. In choosing eggs for pairing, on a purely chance basis, the random combinations made are exactly analogous to those resulting if playing cards are well shuffled and drawn with reference to combinations of red and black. As a matter of curiosity, such a drawing of cards was made to the number of 500 pairs, that being approximately the total number of operations performed in this experiment. The result was as follows: RR 121, RB 249, BB 130, which is remarkably close to the 1 : 2 : 1 ratio required by the law of chance. Therefore, of the eighty pairs of operated animals, ignoring for the present the possibility of a selective mortality, about forty pairs should have been ♂ ♀. Lillie has shown that in cattle the ratio of free-martins to unisexual twins fulfills the 1 : 2 : 1 ratio reasonably well, and this is substantiated by Gowen ('22), who shows, moreover, that in other animals where this ratio is badly deranged—for example, man—the unusual ratio is accounted for by the occurrence of monozygotic twins. Gowen thinks that monozygotic twins occur very rarely in cattle, hence the existence of the 1 : 2 : 1 ratio seems a fact. Such a ratio is quite evident in cases where prepotency ('dominance') of one sex is lacking or incomplete, but if there is complete prepotency of one sex, then the 1 : 2 : 1 ratio is changed to a 3 : 1 ratio favoring the prepotent sex. Such theoretical conditions would require, first, a mechanism, probably physiological, whereby prepotency or predominance could become established, and, second, that this prepotency become effective prior to the beginning of morphological differentiation of sex. The second of these conditions seems

to be fulfilled by this experiment, the first may be plausibly inferred.

But if the ratio of males to females in the paired animals of this experiment does not conform to the mathematical expectation, 1 ♂ ♂, 2 ♂ ♀, 1 ♀ ♀, because of the absence of heterogeneous (male-female) pairs, neither does it approach even remotely the 3:1 ratio to be expected if there were a prepotency constantly favoring a given sex. The only ratio which the figures (44 ♂ ♂, 36 ♀ ♀) reasonably approach is the 1:1 ratio characteristic of normal animals. The only inference to be drawn from this fact alone is that *there is no prepotency which constantly favors a given sex.* This explanation seems at first thought not very satisfying, yet let us see whether there is not something to support it. First, it is in accord with the fact that the study of normal differentiation shows no marked priority of development in point of time favoring either sex. If there is any priority at all, it would seem to favor the female, but the numerical preponderance though small, actually favors the male.

Newman ('23), in "The Physiology of Twinning," discusses the results of asymmetrical vascular anastomoses in the placentae of twins and consequent unequal distribution of blood. Such a condition is almost always favorable to one individual, whose general development is accelerated at the expense of the other. The type of anastomosis and the manner of occurrence do not apply to the present case, but the effect of an unequal distribution of blood might well be the same. The nature of the vascular connection in this experiment is described elsewhere in this paper and it is possible that unequal distribution of blood may occur in many cases. Clearly, the operation of this factor would not consistently favor either sex and, therefore, might tend to equalize the sex ratio.

Such a condition, however, should be revealed through cases of unequal growth, and observation and measurements fail to show this. Pairs are almost always of very uniform size (p. 54 and pl. 6). Again, the result of such a condition

should appear in the gonad itself, in cases of tardy or incomplete differentiation of one member. Such cases are not as yet found. Whether this cause can effectively permit an earlier differentiation of sex in one twin, yet be of a degree insufficient to effect the general well-being and growth, is questionable. A further examination of this point is desirable.

In his study of the free-martin, Lillie assigns constant prepotency to the male on the basis of marked priority in the development of the interstitial tissue (Bascom, '23), and this reason does not apply to this experiment. May it not be, then, that prepotency is established, for each individual case, not regularly, but by the accidents of development which may give either one or the other of the two united individuals the advantage irrespective of their sex. This would imply a very equal balance of the opposing quantitative determiners from the very beginning, and this is just what we should expect theoretically in a ♂ ♀ combination. Obviously, we are not concerned for ♂ ♂ and ♀ ♀ pairs. For convenience, let us assign, as did Goldschmidt, absolute values to the sex-determining factors expressed in a Mendelian formula for sex, bearing in mind that in this case there is no variable.[1] We may represent the female by the formula (MM)FF and the male by (MM)FO. It is only necessary to assume that (MM) is always greater than FO but less than FF, or, expressed serially FF>MM>FO. Any numbers that maintain the value of (MM) at a point about equally distant between FF and FO will serve to demonstrate. Suppose that M=30

[1] The evidence is good that there is a typical sex-chromosome mechanism in urodeles. King ('12), in Necturus, describes a body attached to a multiple chromosome, which behaves throughout both maturation divisions as a typical x-chromosome; and Parmenter ('19) suggests a similar condition in Amblystoma tigrinum. Also there is reason for believing that in Amphibia, at any rate in Anura, the female is the heterogametic sex, since Loeb obtained frogs of both sexes from parthenogenetic eggs. However, data secured by Witschi ('23), from breeding and self-fertilization experiments with an hermaphroditic frog, indicate that the female is homogametic as in most animals. The question is not essential, and we have used the conventional formula with the female homogametic.

and F=40. Then (MM)60>(FO)40=♂, and (MM)60<(FF)80=♀. Now let us see what happens in a combination where all factors are operating en masse, that is, all hormones are mingled in a common blood stream. Then we have $\frac{(MM)}{(MM)}$ 120 = $\frac{(FO)}{(FF)}$ 120, or an approximate equalization of the total quantitative value of the sets of opposing factors. This tends to establish in a two-sexed pair of embryos a condition of close balance or unstable equilibrium—an equilibrium that might well be overbalanced in any given case by a small quantitative variation, a variation not constant for either sex. Each case is then decided quite arbitrarily, which in the end would result in a rather impartial distribution of the heterozygotic pairs. It is perhaps in this way that the close approximation to a 1:1 ratio is to be explained.

However, it may not be convincingly clear how such a condition of near-equilibrium can be decisively broken, but this is not difficult to understand in the light of an 'all or none reaction' which has been advisedly proposed. This type of response is a common physiological phenomenon, and there is real evidence that the hormones of sex act in this manner. Both Lillie and Goldschmidt invoke this sort of response to explain the definite demarkation between male and female parts in organs that are 'intersexual.' Goldschmidt explains that only in a macroscopic way are the various organs of Lymantria intersexual, that in reality they constitute a mosaic of characters, each individual for its proper sex; *showing a very sharp transition from the period of influence of one hormone into that of the other.* This presupposes a full and entirely independent action of each hormone, with a very sudden passage through a point of near-equilibrium. Lillie ('23) also suggests that the action is an 'all or none response' and that the important factor is time of onset rather than duration of influence. The work of Lipschütz, Pézard, and others has shown that a very small fragment of testicular tissue is quite sufficient to maintain fully the secondary sex characters of various mammals and birds. This fact has been noted by almost all who have worked at castration and

transplantation of gonads. Lipschütz ('21) finds that a surprisingly small 'minimum' is fully as effective as the full secretory output of the gland. Pézard ('20) has demonstrated this still more nicely in his study of the cock, where a very small fragment of testis is capable of maintaining the elaborate secondary sex characters of the bird at their full development, but with the passing of a certain minimum the characters disappear completely. *There is no intermediate condition, a certain minimum amount becomes completely effective at a definite threshold.* From these facts Pézard has formulated 'an all or none law' of action for the hormone.

To sum up, we may say that the 'all or none law' is a well-known type of physiological response; that there is strong evidence that many hormones, including those for sex, operate in accordance with this law, and that, in the present experiment, the apparent absence of a prepotency that constantly favors either sex may be thus explained. Starting from a state of near-equilibrium, it is quite conceivable that a small initial advantage gained in either direction might, by a cumulative impetus, move rapidly to a decisive point. In the course of development the time that elapses between the formation of the 'indifferent' definite gonad and the beginning of sex differentiation seems, moreover, quite adequate for such an adjustment to be effected.

SUMMARY

1. Embryos of Amblystoma punctatum were joined in parabiosis at a stage shortly after closure of the medullary folds. Eighty pairs survived until sex was identifiable histologically, and invariably members of a pair were of the same sex. The ratio of the sexes in these pairs approaches the 1:1 ratio found in normal animals. There were 44 ♂ ♂ pairs and 36 ♀ ♀ pairs.

2. Comparison of the gonads of paired animals, with a series of gonads from normal animals, shows that development is apparently normal in all respects. No indication of an intersexual condition appears.

3. The law governing chance combinations requires, in a sufficiently large number of cases, this ratio: 1MM:2MF:1FF. Thus, approximately half the pairs should represent what were originally ♂ ♀ combinations. Unless a selective mortality has occurred, reversal of sex must be assumed. In joining pairs, the precaution was taken to choose members from different bunches of eggs.

4. The 1:1 ratio approximated in paired animals indicates that there is no prepotency constantly favoring either sex, since, in such case, a 3:1 ratio would be expected, favoring the given sex.

5. In establishing a random prepotency from a state of near-equilibrium, a response of the 'all or none' type is suggested.

6. The theory of a selective mortality seems unsupported. Although this explanation cannot be absolutely rejected, the evidence is strongly against it.

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All figures on plates 1 to 5 are magnified 320 diameters.

PLATE 1

EXPLANATION OF FIGURES

1 Cross-section through germ-cell region of normal embryo of Amblystoma punctatum, stage 36, 6 mm. *NCH*, notochord; *ARCH*, archenteron; *WD*, Wolffian duct; *PGC*, primordial germ cells. About six days after operating stage.

2 Cross-section normal embryos, stage 45+, 10 mm.; showing 'paired anlagen' of germ glands. *NCH*, notochord; *AO*, aorta; *WD*, Wolffian duct; *MNP*, median plate of lateral mesoderm; *KDT*, kidney tubule; *PRA*, the 'paired anlagen.'

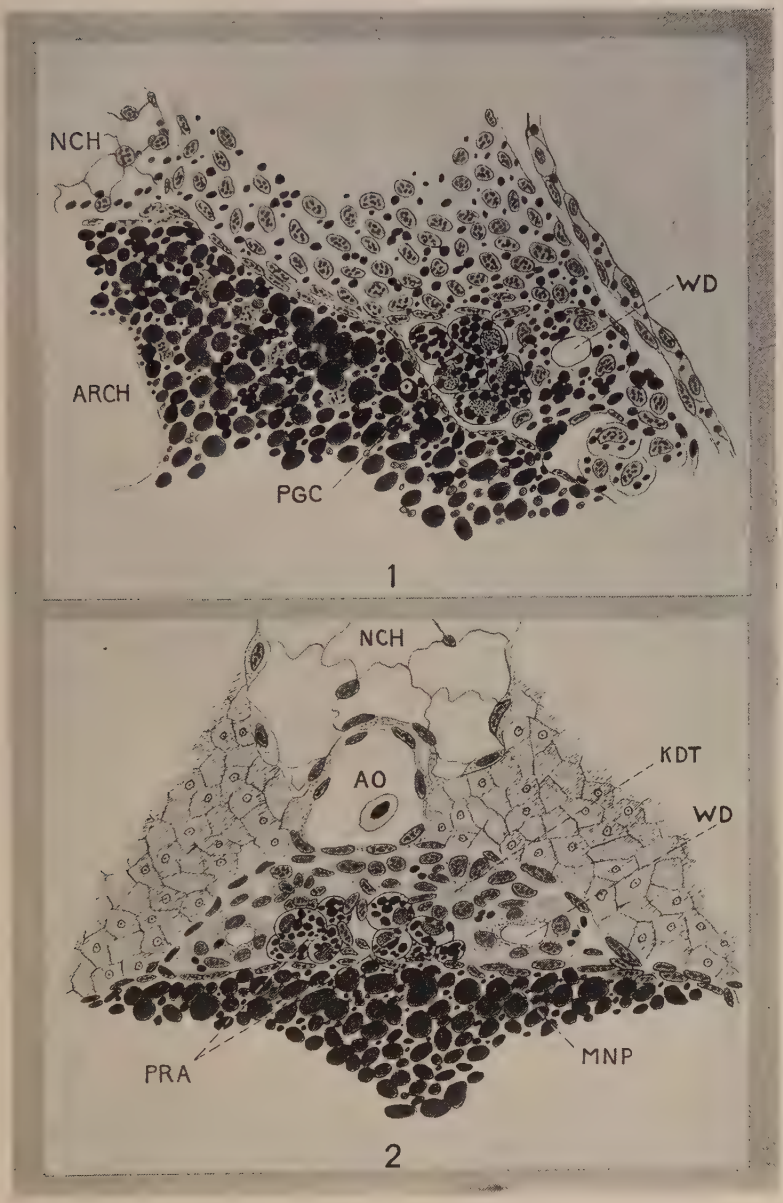

NCH
WD
ARCH
PGC
1
NCH
KDT
AO
WD
PRA
MNP
2

PLATE 2

EXPLANATION OF FIGURES

3 Embryo, series III, May 15, 14 mm. Cross-section through germ ridges. *WD*, Wolffian duct; *KDT*, kidney tubules; *INT*, intestine; *MES*, mesentery; *GMR*, germ ridges.

4 Embryo, series VI, 16 mm. Later stage, gonad pinched off from kidney, fat-body forming. *VC*, vena cava; *KDT*, kidney tubules; *MES*, mesentery; *PRT*, peritoneum; *FB*, fat-body.

5 Embryo, series VI, 18 mm., cross-section through 'indifferent gonad.' *VC*, vena cava; *KDT*, kidney tubule; *MES*, mesentery; *PRT*, peritoneum; *FB*, fat-body; *SXC*, sex-cord material. This animal on verge of differentiation.

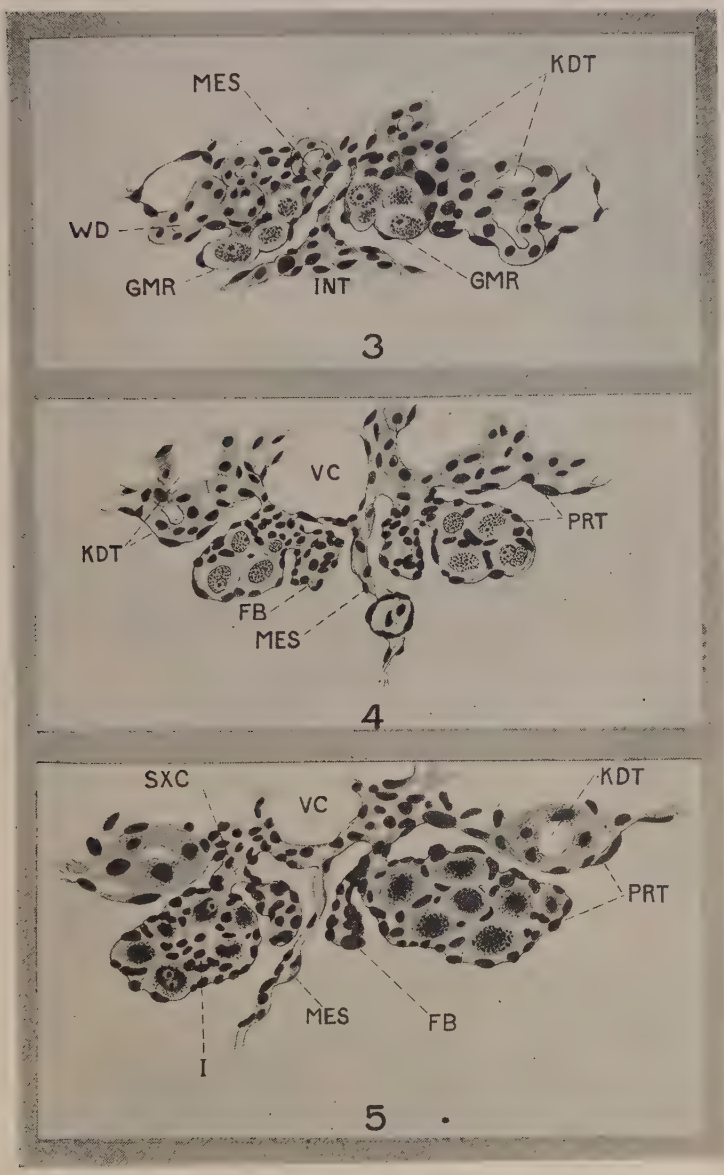

MES
KDT
WD
GMR
INT
GMR
3
VC
KDT
PRT
FB
MES
4
SXC
VC
KDT
PRT
MES
FB
I
5

PLATE 3

EXPLANATION OF FIGURES

6 Normal larva, 18 mm. Showing testis, early stage of differentiation. Note that spermatogonia resemble very closely primordial germ cells. *VC,* vena cava; *WD,* Wolffian duct; *MD,* Müllerian duct; *FB,* fat-body.

7 Normal larva, September 15th. Showing later stage of testis. Note small increase in size, and formation of longitudinal collecting duct, and vasa efferentia. Also notice appearance of lipoid in fat-body. *WD,* Wolffian duct; *MD,* Müllerian duct; *FB,* fat-body; *SXC,* sex-cords; *I,* interstitium; *LCD,* longitudinal collecting duct; *VEF,* vasa efferentia.

8 Normal larva, October 15th. Testis at stage of metamorphosis. Note formation of nests of spermatogonia, separated by lamellae of interstitial cells. *WD,* Wolffian duct; *MD,* Müllerian duct; *KDT,* kidney tubule; *LCD,* longitudinal collecting duct; *SXC,* sex-cord; *I,* interstitium.

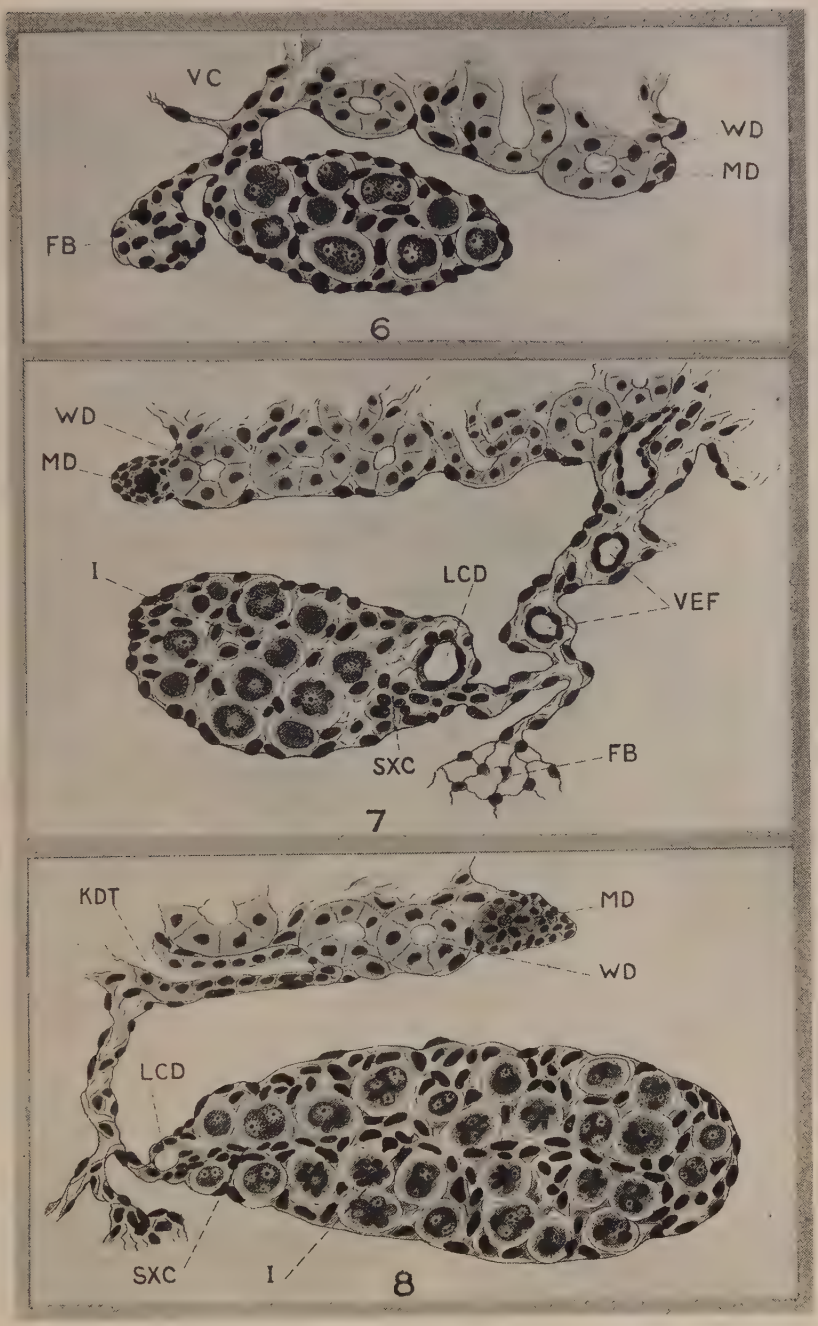

VC
WD
MD
FB
6
WD
MD
I
LCD
VEF
SXC
FB
7
KDT
MD
WD
LCD
SXC
I
8

PLATE 4

EXPLANATION OF FIGURES

9 Normal larva, July 15, 21 mm. Early stage of ovary. Note ovarian cavity and characteristic nuclear structure. *VC*, vena cava; *FB*, fat-body; *SXC*, sex-cord material; *OVC*, ovarian cavity.

10 Normal larva, July 25th, 18 mm. More advanced ovary. Note marked growth of some of the oogonia. *WD*, Wolffian duct; *VC*, vena cava; *MES*, mesentery; *FB*, fat-body; *OVC*, ovarian cavity.

11 Normal larva, July 22nd, 20 mm. Ovary, showing farther growth of oocytes with encroachment upon ovarian cavity. Note rudiment of *MD*. *GL*, glomerulus; *YOO*, young oogonium.

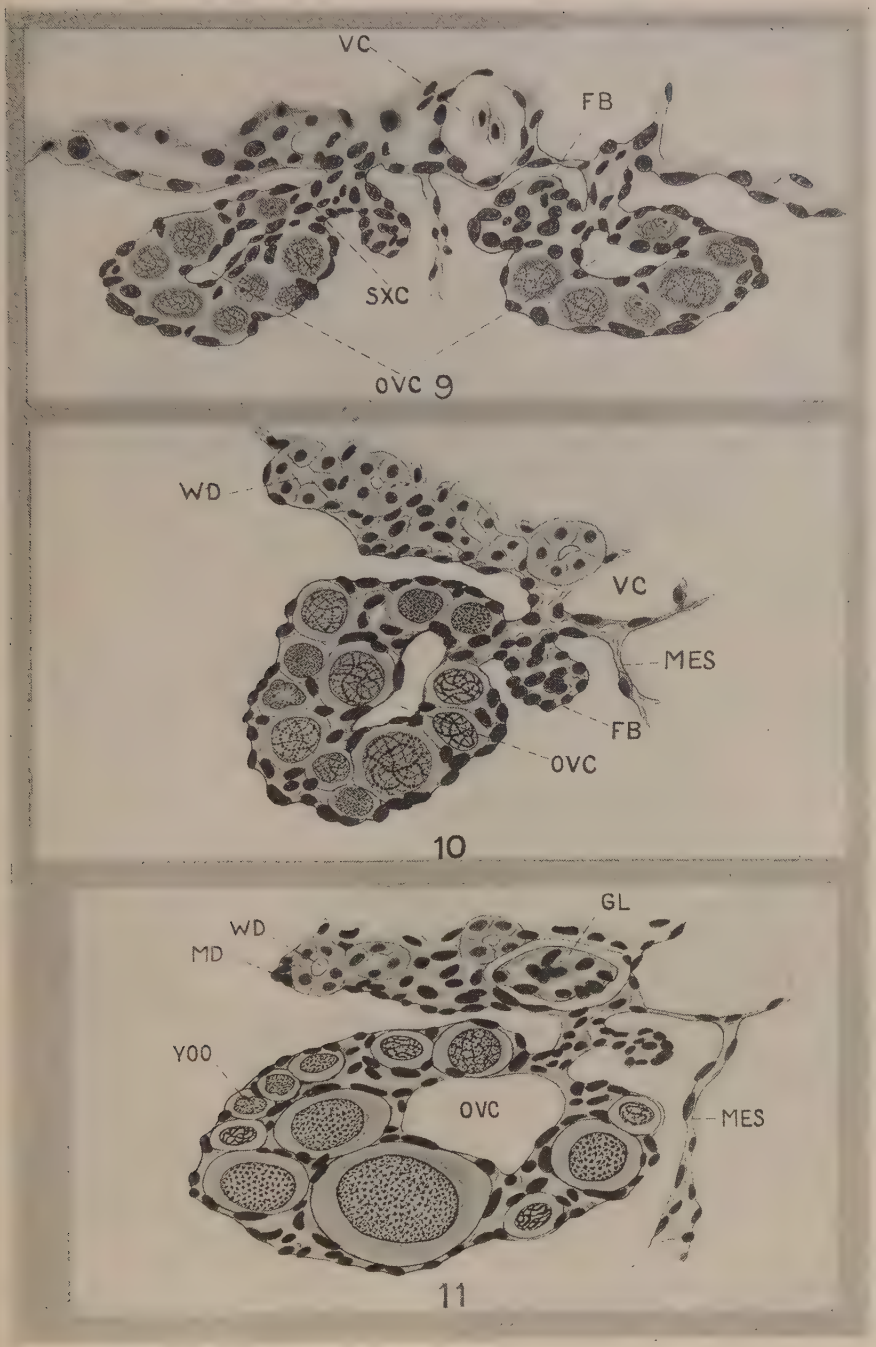

VC
FB
SXC
OVC
9
WD
VC
MES
FB
OVC
10
GL
WD
MD
YOO
OVC
MES
11

PLATE 5

EXPLANATION OF FIGURES

12 Normal larva, October 25th. Well-developed ovary, showing obliteration of ovarian cavity through growth of oocytes. Note farther growth of *MD* and compare with males. *I*, interstitium; *YOO*, young oogonia.

13 Larva, series III. November 20th, 26 mm. Farther advanced stage of ovary, about time of metamorphosis. A typical, but not a large ovary.

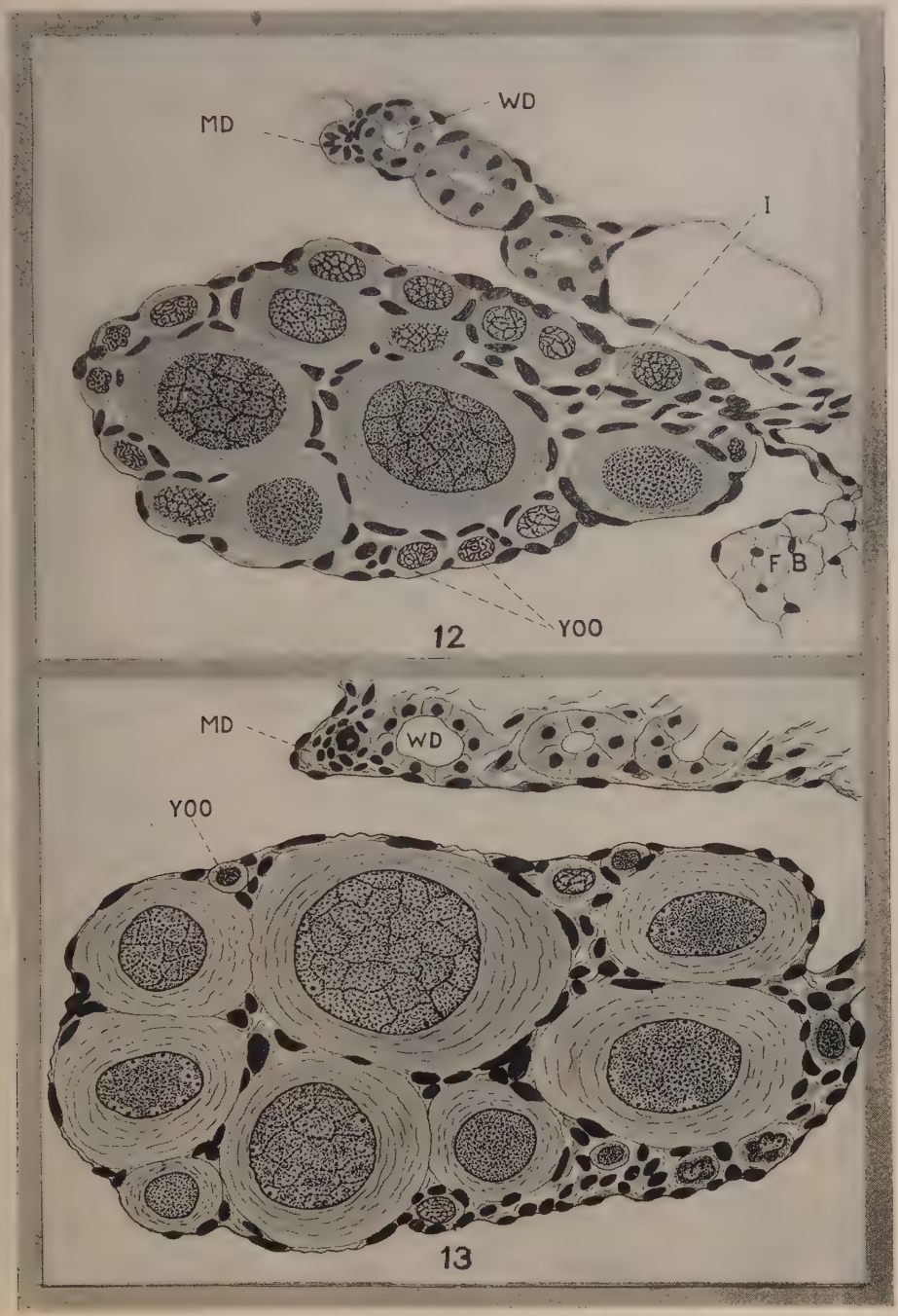
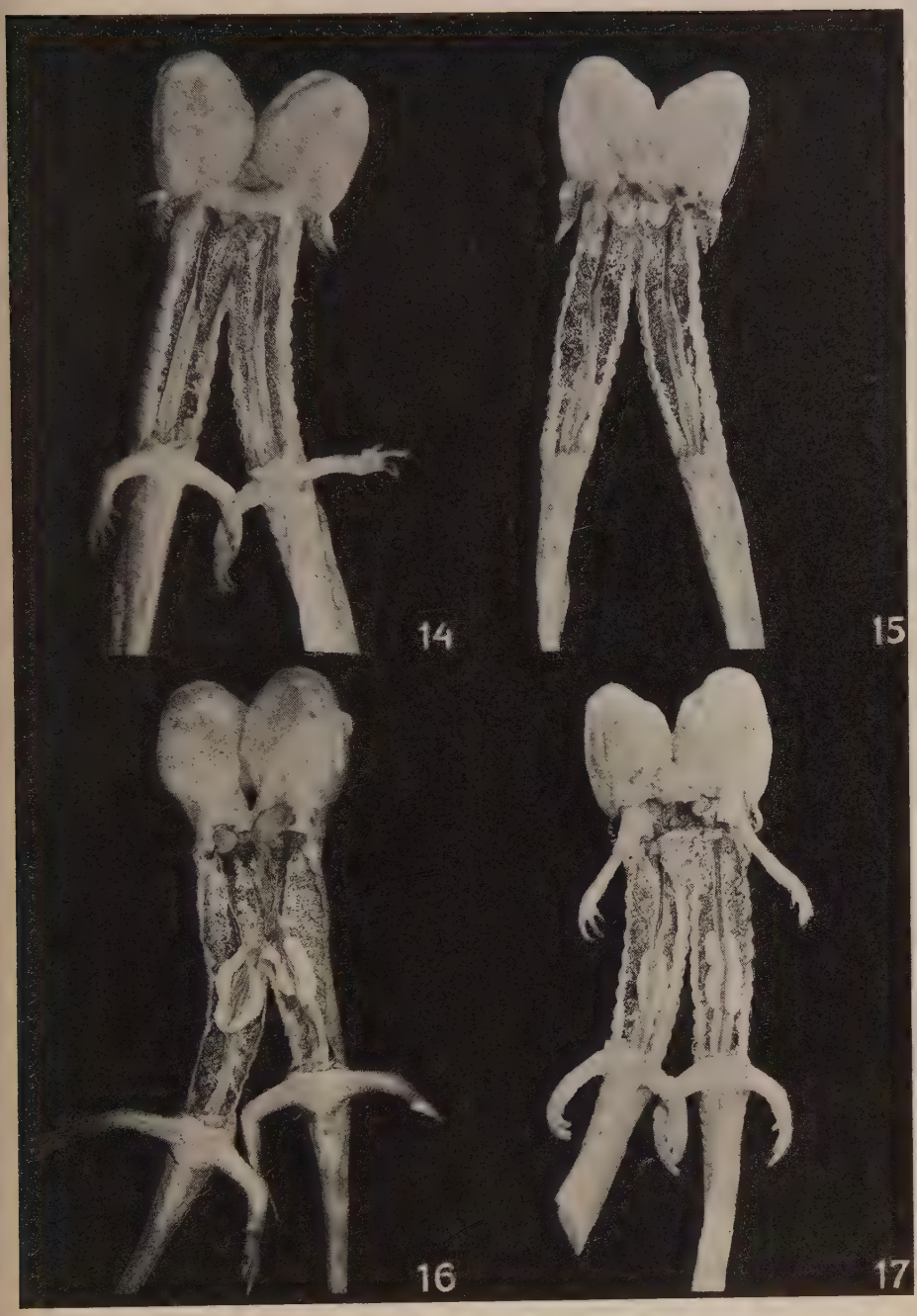

MD
WD
I
F B
YOO
12
MD
WD
YOO
13

PLATE 6

EXPLANATION OF FIGURES

14 and 15 Photographs of dissections of typical ♂♂ pairs, about stage of metamorphosis. October 15th. Testes may be seen as very small slender white bodies lying on top of kidneys. Note also the Müllerian ducts extending anteriorly from kidneys and compare with photographs of females. × 2½.

16 and 17 Photographs of dissections of typical ♀♀ of the same stage. The remarkable differences are evident. × 2½.


14
15
16
17

Reprinted from The American Naturalist, Vol. LIX,
May–June, 1925

THE PHYSIOLOGICAL SIGNIFICANCE OF CONJUGATION AND ENDOMIXIS IN THE INFUSORIA[1]

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The first serious attempt to give a comprehensive survey of the Protozoa is afforded by the studies of Otto F. Müller made during the latter part of the eighteenth century, though it is true that Dr. John Hill in his "History of Animals," published in 1752, stated that "their want of magnitude does not exclude them from their rank among animated beings . . . I have arranged them into a regular method, and given them denominations"—and one denomination he coined was Paramecium. But Müller, profiting by the microscopical studies in the interim, as well as by the establishment of the Linnaean system of classification, produced in his Animalcula Infusoria the actual base line for future investigations. Our present interest, however, in Müller's work is due to the fact that he rightly interpreted pairs of Infusoria as undergoing a sexual process and not as dividing longitudinally. The latter, strange to say, was the view of his successors down to 1858, when Balbiani published his observations on the existence of sexual reproduction in the Infusoria.

Balbiani thought that the nucleus and micronucleus became transformed into sexual organs—the macronucleus producing eggs, and the micronucleus, sperm—and he thereby obscured a minimum of fact with a plethora of imagination. Nevertheless, with Balbiani's work as the point of definite departure, the problem at the hands of Bütschli and Engelmann in 1876 was placed well upon the road toward its present-day development. These authors established the dimorphic nature of the nuclear

[1] Lecture delivered at the Woods Hole Marine Biological Laboratory, July 18, 1924.

apparatus of the Infusoria and the formation of a new macronucleus from the micronucleus during conjugation, though it remained for a series of workers during the eighties of the last century—notable among them being Balbiani, Maupas and Richard Hertwig—to observe the important cytological features of conjugation, including synkaryon formation, in the Protozoa.

But both Bütschli and Engelmann did more than supply cytological data: they offered the first concrete suggestions in regard to the significance of fertilization. Bütschli believed it to be a process of rejuvenation, and Engelmann one of reorganization: a difference in details, but an agreement in fundamentals. Both thought that continued reproduction by division tends to slow down through loss of vitality, and that fertilization restores the pristine vigor of the cells. And thus the so-called "dynamic" interpretation of fertilization phenomena in the Protozoa arose.

These pioneer studies of fertilization in the Protozoa were made during and, it is probably fair to say, under the stimulus of the classic period of cytological advance. Bütschli and Auerbach, Oscar Hertwig and Fol, Flemming, van Beneden and Boveri were establishing the essential features of mitosis, maturation and fertilization in metazoan eggs which gradually led to the full realization of the genetic continuity of the germ nuclei foreshadowed a generation before. And this, in turn, formed the indispensable prelude to the elucidation of the chromosome mechanism which cytologists have supplied to geneticists as the physical basis of heredity.

Thus was added content to the view, emphasized two centuries before by Harvey and modernized by Oscar Hertwig and Minot, that fertilization supplies a stimulus to egg development, and to the equally ancient and probably more important one that fertilization affords the basis of biparental inheritance in all sexually reproducing organisms.

The answers to several fundamental physiological problems have been sought in the study of the Infusoria,

which may be stated in the form of two general questions:

(1) Do these organisms possess the power to reproduce indefinitely by division, under favorable environmental conditions, without recourse to conjugation or to endomixis?

(2) In cultures which are exhibiting lowered vitality, does either conjugation or endomixis restore the normal metabolic condition and "rejuvenate" the organisms?

We shall consider these and attempt to present definite answers, but it is unnecessary to state that since there are always at least two sides to every question, impartial as the reviewer wishes to be, the personal equation must perforce find expression.

The first consistent investigation of the power of the Infusoria to reproduce indefinitely without recourse to conjugation is afforded by the elaborate studies of Maupas in which he presented a wealth of data and a series of interpretations that for more than a decade were taken at their face value and believed to prove that the life history runs in cycles. In brief, a long period of multiplication by cell division is succeeded by an epidemic of conjugation, which inaugurates a new cycle, and is comparable in its physiological aspect with the period of sexual maturity in the metazoa. If conjugation does not occur, the race rapidly degenerates and dies, and accordingly conjugation counteracts the tendency to senile degeneration and causes rejuvenescence.

Now to-day we are asking whether Maupas's data show what he says, and whether he says what he means! This is not merely of historic interest because his hundreds of pages filled with observational and experimental details have had great weight.[2]

Maupas definitely says, "My cultures have experimentally shown that these Protozoa do not escape the general law of senescence. They exhaust themselves and become old by the exercise of their functions, or, in other words,

[2] Calkins, "What did Maupas mean," AMER. NAT., 57, 1923.

because of a necessity inherent in their structure and constitution." This, as Calkins points out, is exceedingly explicit and is the burden of Maupas's entire experimental study.

But Maupas states that his records indicate that "the power of reproduction follows no such diminishing and parallel course. It is maintained almost intact even a long time after the other functions and the entire organism are shown to be greatly reduced by senile degeneration . . . the Infusoria, although feeble and much reduced in size, continue to divide with almost the same rapidity as at the beginning." In other words, Maupas apparently believed that progressive physiological and morphological degeneration is compatible with a normal division rate. As Calkins's analysis has shown, Maupas's own data do not indicate the biologically untenable conclusion that division rate, which subsequent investigation has amply demonstrated is a most subtle index of metabolism, is unaffected by degenerative changes.

So, although we can at least accept it as a fact that Maupas found progressive degeneration as cell division proceeded, we must reject his statement that the division rate was unaffected. His studies indicate cyclical change, both structural and physiological, during interconjugation periods. And thus the so-called cycle definitely entered the literature on the Infusoria.

Without minimizing the importance of the pioneer work of Maupas, it is nevertheless true that his method of culture was inadequate to the problem, and the great advance which paved the way for all more recent work was the adoption of the daily isolation method by Calkins when he took up the problem after Maupas's results had been accepted for over a decade. This method rendered possible careful control of the culture medium from day to day, accurate records of the rate of division and the positive elimination of conjugation. In brief, it afforded for study precise pedigree cultures of Infusoria.[3]

[3] Calkins, "Studies on the life history of Protozoa. I. The life cycle of Paramecium caudatum," *Arch. f. Entw-mech.*, 15, 1902.

Calkins's investigations on the question of the cyclical nature of the life history of Infusoria began in 1901 with Paramecium caudatum and have been continued on various species, culminating in an extensive analysis of the life history of Uroleptus mobilis which is still in progress. His answer to the question under consideration is that the "physiological processes of metabolism are not capable of unlimited activity . . . there is a progressive weakening of metabolic vigor from an optimum shortly after conjugation." "Such weakened protoplasm, if not allowed to conjugate, inevitably dies, as does the somatic protoplasm of metazoa." "Natural death from old age . . . is actually inherent in protoplasm as little differentiated as in these single-celled animals."[4]

But other workers, following the lead and using, in general, the technique of Calkins, have reached other results and conclusions. Enriques has bred cultures of several Infusorians, notably Glaucoma pyriformis, for nearly 3,000 generations, without signs of lowered vitality. At New Haven, Paramecium aurelia has been carried for some fifteen years and more than 10,000 generations, and, among others, Spathidium spathula for over a year, during which 1,000 generations were attained. Conjugation has not occurred in the direct line of any of the cultures. Similar results have been attained by Baitsell on Pleurotricha lanceolata and Oxytricha fallax, by Mast on Didinium nasutum and by Dawson on Oxytricha hymenostoma.

It is highly significant that the longevity of Infusoria —the so-called life cycle—has been gradually though consistently prolonged year by year as pedigree culture technique has been perfected. Even such relatively ubiquitous forms as Paramecium aurelia and caudatum, which are able to survive temporarily under very diverse conditions, gradually succumb to prolonged subjection to media which at first seem entirely suitable. Ample evi-

[4] Calkins, "Uroleptus mobilis, II, Renewal of vitality through conjugation," *J. Exp. Zool.*, 29, 1919.

dence of this is afforded by the history, during more than threescore years, of the study of Paramecia cultures.

Quite a similar history has been briefly recapitulated at New Haven in the study of Spathidium spathula during the past four years. The majority of the cultures showed what heretofore would have been interpreted as inherent "cycles" which culminated in death unless conjugation was permitted, but the drift toward longer life of certain cultures as the experiments progressed, and finally the attainment, as just mentioned, of more than a thousand generations with every indication of the potentiality to continue to divide indefinitely, can, we believe, be reasonably interpreted in but one way.[5]

The only species extensively bred which still proves refractory is Uroleptus mobilis. The extreme rarity of this species may reasonably, we believe, be attributed to peculiar conditions of life demanded, and its cycle to the fact that some crucial factors of the milieu have thus far not been supplied. If this is not the proper interpretation of the cyclical character of the Uroleptus life history, then this form certainly suffers a limitation which is not shared by the other species investigated.

To one question, then, we believe an unequivocal answer has been given—the Infusoria in general can, under favorable environmental conditions, reproduce indefinitely by division without recourse to conjugation. The cycle is a phantom which is slowly but surely receding toward the vanishing point, as the special requirements of particular organisms are being established.

From the study of various species of hypotrichous Infusoria as well as from the long continued Paramecium aurelia culture at New Haven, it was found that minor periodic fluctuations of the division rate occur, from which recovery is autonomous. These fluctuations were termed rhythms and contrasted with the so-called

[5] Woodruff, "The present status of the long continued pedigree culture of Paramecium aurelia at Yale University," Proc. Nat. Acad. Sci., 7, 1921.
Woodruff and Moore, "On the longevity of Spathidium spathula without endomixis or conjugation," Proc. Nat. Acad. Sci., 10, 1924.

"cycle," which comprises a varying number of rhythms and, according to Calkins, ends in the death of the race if conjugation or artificial stimulation is not resorted to.[6]

An intensive study of rhythms was made in Paramecium aurelia which showed that subjection of the culture to the most constant environmental conditions failed to eliminate the rhythms, but, instead, they appeared still more pronounced. It was also found from a study of the temperature coefficient of the rate of division that this is influenced by temperature at a velocity similar to that for a chemical reaction, except when the rhythms interfere. Thus it became apparent that there are inherent rhythmical changes in the phenomena of the cell which produce slight fluctuations in the rate of division.[7]

Daily cytological study of pedigree Paramecium aurelia and caudatum showed that the rhythms in these species are coincident with a nuclear reorganization process which was termed endomixis. It will be recalled that an essential difference between endomixis and conjugation is the absence of the third micronuclear division, which in conjugation forms the gametic nuclei, and, of necessity, the non-formation of a synkaryon.[8]

This process of endomixis—or, if you will, parthenogenesis, though we believe the non-committal term preferable at the present stage of our knowledge—has also been found in one other species of the genus Paramecium, P. polycaryum; Calkins has found it in Didinium nasutum and Uroleptus mobilis, and Moore in Spathidium spathula. Fermor described an internal reorganization process during encystment in Stylonychia pustulata which, however, involves a nuclear fusion and is, therefore, not endomixis but autogamy.[9]

[6] Woodruff, "An experimental study on the life history of hypotrichous Infusoria." *J. Exper. Zool.*, 2, 1905.

[7] Woodruff, "The influence of general environmental conditions on the periodicity of endomixis in Paramecium aurelia," *Biol. Bull.*, 33, 1917.

Woodruff and Baitsell, "The temperature coefficient of the rate of reproduction of Paramecium aurelia," *Amer. J. Physiol.*, 29, 1911.

[8] Woodruff and Erdmann, "A normal periodic reorganization process without cell fusion in Paramecium," *J. Exp. Zool.*, 17, 1914.

[9] Fermor, "Die Bedeutung der Encystierung bei Stylonychia pustulata," *Zool. Anz.*, 42, 1913.

The discovery of endomixis in Paramecium aurelia and caudatum coincident with rhythms which are due to intrinsic and not external factors naturally led to the interpretation of endomixis as the phenomenon involved—a rhythm thereupon being regarded as the outward physiological expression of the underlying cytological phenomena of endomixis. Furthermore, it was shown in Paramecium aurelia and caudatum that cessation of endomixis is soon followed by the death of the culture. This affords the best possible proof that a periodic occurrence of endomixis is indispensable for the continued life of these species.

So far as Paramecium aurelia and caudatum are concerned, the rhythm may perhaps be considered as an expression of a sort of temporary "senescence" for which is provided an internal, automatic antidote in the form of endomixis. But I would emphasize that this is a case of trying to force new wine into old bottles in order to save an idea. One certainly must grant that the original idea of senescence in Infusoria is far removed from this subtle, automatically arrested type which rhythms may indicate. To shrink the cycle to the rhythm is to annihilate the cycle.

But we are not interested in an academic discussion of terms—the fact remains that still more recent work has shown that in other species of Infusoria endomixis does not occur during the free life of the cell, though rhythms, as originally defined, are present. This is the case in Spathidium spathula—endomixis occurring, as Moore has shown, during encystment; and in Paramecium calkinsi, in which endomixis, as demonstrated by Spencer, does not take place at all. In other words, endomixis occurs at the low point of the rhythms in Paramecium aurelia and caudatum, and though, from the very nature of the process, it accentuates the drop in the division rate, nevertheless it is not the underlying factor of rhythms after all—for rhythms exist *without* endomixis in other species.

Such being the case, the whole problem takes on another aspect: we have two phenomena to consider—rhythms and endomixis. For the moment, however, we are seeking a definite answer to the specific question whether Infusoria can reproduce indefinitely by division without endomixis. It is clear that Paramecium aurelia and caudatum can live indefinitely without conjugation, but that suppression of endomixis inevitably is followed by death, and accordingly endomixis is indispensable in the life history of these species. But Enriques states that endomixis did not occur in his long-continued Glaucoma culture, and at New Haven it has been shown that Paramecium calkinsi, which does not undergo endomixis, apparently can reproduce indefinitely by division, and that Spathidium spathula, which undergoes endomixis solely while encysted, can reproduce indefinitely by division without encystment and, therefore, without endomixis.[10]

Accordingly, although the suppression of the endomictic process while in progress in the free life of Paramecium aurelia and caudatum is fatal, the undoubted fact that in other species of Infusoria it may be eliminated by preventing its onset (*e.g.*, by preventing encystment) and in still other species it apparently does not occur at all, forces us to the definite conclusion that endomixis is not a fundamental, indispensable factor in the life history of the Infusoria—that they can reproduce indefinitely by division under favorable environmental conditions.

In brief, the time-honored question whether the Infusoria degenerate and die when conjugation is prevented has, we believe, been answered unequivocally in the negative, and likewise, the very new question whether Infuso-

[10] Enriques, "Duemila cinquecento gencazioni in un infusorio, senza coniugazione nè partenogenesi, nè depressioni." R. Acad. Sci. Bologna, 1916.

Moore, "Endomixis and encystment in Spathidium spathula," *J. Exp. Zool.*, 39, 1924.

Spencer, "Studies on Paramecium calkinsi," *J. Morph. and Physiol.*, 39, 1924.

ria in general degenerate and die when endomixis does not take place has also been answered unequivocally in the negative. Therefore, not only is synkaryon formation unnecessary, but a periodic reconstitution of the nuclear apparatus from the micronucleus is unnecessary. All the evidence cumulatively points to the conclusion that the zygotic nucleus constituted during syngamy and the nucleus constituted during endomixis in the Infusoria are adequate for indefinite division of the cell under favorable environmental conditions.

Having eliminated, to our satisfaction at least, amphimixis, endomixis and the cycle we still have the rhythm. To what are rhythms due? Defined from a study of the life history of hypotrichous Infusoria in 1905 as "a minor periodic rise and fall of the fission rate, due to some unknown factor in cell metabolism, from which recovery is autonomous" they have since then been in evidence in every culture of Infusoria whose division rate has been published. Endomixis in Paramecium aurelia and caudatum was believed to offer the solution, but it is evidently not the cause of the rhythms—though it accentuates them in these species—since our belief that endomixis would be found during the rhythms of every species has not proved to be correct. We are forced, therefore, to fall back for the present on our general statement of twenty years' standing—"due to some unknown factor in cell metabolism."

But there is one possibility which, remote as it is, must not go unmentioned. Rhythms in Infusoria synchronous with endomixis may not be the same phenomenon as rhythms in forms which do not undergo an endomictic process. Although the term was originally applied to fluctuations in the fission rate of species which subsequent work has shown do not have endomixis, and then applied to fluctuations in the rate of Paramecium aurelia which later work has shown does undergo endomixis, it is a fact that the most extensive attempts to eliminate these fluctuations by constant environmental conditions were made

on Paramecium aurelia before endomixis was known. An equally intensive study of a form without endomixis might show that the rhythms can be eliminated, although Spencer has already carried on a series of cultures of Paramecium calkinsi for this particular purpose which makes it almost certain that the rhythms are inherent.

We have considered the question whether conjugation is a necessary factor in the life history of the Infusoria and have answered it in the negative. But, obviously, because conjugation is not necessary under favorable environmental conditions, it does not follow that it is not necessary under other conditions—that it does not have a dynamic function which is brought into play when the organism meets various vicissitudes of its environment.

In opposition to the prevailing view based chiefly on the work of Bütschli, Maupas insisted that conjugation does not increase the rate of reproduction: it merely wards off senile degeneration when it occurs before this is far advanced. Richard Hertwig from very limited experimental evidence reached similar results, except that he found after conjugation the division rate was *lower* than before—and so he interpreted the rejuvenating effect of conjugation as due to a repression of division energy.

However, the work which is significant to-day begins with Calkins's studies carried out twenty years ago on Paramecium caudatum. In degenerating pedigree cultures of this organism, he found that so-called rejuvenescence occurs after exogamous or endogamous conjugation, but only in a small percentage of cases, for only a few unions result in continued life of the exconjugants. These failures, he thought, indicate that only certain combinations are capable of yielding fertile results, especially in view of the fact that he obtained a high percentage of viable conjugants from wild Paramecia. From further work on the same organism he concluded that conjugation between two cells results in the complete reinvigoration of all activities, both physiological and germinal.

So the problem stood when Jennings in 1913 published his extensive study on the effects of conjugation in Paramecium, in which he marshalled the data from Maupas and Hertwig to support his own brilliantly attained result, from experimental and biometrical analysis, that "the average rate of reproduction is less after conjugation than before." That is, when he took, for example, two sets of animals of the same stock and history, both ready to conjugate; permitted one set to conjugate and prevented the other, he found that those which had conjugated divided thereafter on the average less rapidly than the others. In most cases the rate of fission was very considerably greater in those that had not conjugated, and in no cases did the conjugants have a higher rate of fission. Furthermore, as a rule mortality was much higher, under the same conditions, among animals that had conjugated than among those which had been prevented from conjugating—regardless of whether the animals were unrelated or were members of the same race. So Jennings naturally asked: "Is it not time that the statements or implications that in the Infusoria conjugation results in increased reproduction should disappear from the literature of science?" And four years later Mast's studies on Didinium nasutum led him to agree that conjugation has no appreciable effect on the death rate, fission rate or variation in the fission rate.[11]

In 1917 Calkins resumed his study of the problem, employing a pedigree culture of the hypotrich Uroleptus mobilis. Carrying more than thirty series from exconjugants, he found that there was a progressive weakening of metabolic vigor from an optimum shortly after conjugation. This progressive weakening he attributes, as we have stated, to inherent senescence, while we would attribute it, as we have our earlier results with Spathidium, to extrinsic causes. However the condition arose, the significant fact for the moment is that the animals

[11] Jennings, "The effect of conjugation in Paramecium," *J. Exp. Zool.*, 14, 1913; "Life and Death, Heredity and Evolution in Unicellular Organisms," Boston, 1920.

were transformed from a condition of metabolic weakness to a condition of optimum metabolic vigor by the process of conjugation. Animals which were not allowed to conjugate, under the conditions of the experiment, died.

Now since Jennings showed on a large scale in his studies "that conjugation brings about great variation in vitality and reproductive power" in Paramecium, it is perfectly reasonable to regard single examples of increased fission rate following conjugation as one of the rare extreme variants. But Uroleptus shows almost without exception stimulation following conjugation, so that this explanation is no longer tenable; and Calkins in these experiments has certainly supplied the first apparently unassailable evidence that conjugation is followed by renewal of vitality as indicated by increased reproductive power.

Before 1920 our studies were directed to the question: What happens when conjugation is prevented? But then we turned to the question: What happens when conjugation occurs? Spathidium spathula was selected for the work after a long search for favorable material because it affords the several features which are essential for the successful attainment of the desired data: sufficient size to render practical the daily isolation of large numbers of animals; the non-occurrence of endomixis in free living stages; and, most important of all, the frequent onset of epidemics of conjugation between pedigree animals so that non-conjugant and conjugant lines can be secured and compared at crucial periods. Conjugation could be obtained almost at will by allowing a few animals to remain on a culture slide for a day or two without an adequate supply of Colpidia, which is the staple diet of Spathidium. Incidentally, it may be mentioned that Moody's experience with Spathidium was quite different in that no evidence of ability to conjugate was evident, apparently because it was an amicronucleate race.[12]

[12] Woodruff and Spencer, "Studies on Spathidium spathula, I, The structure and behavior of Spathidium," *J. Exp. Zool.*, 35, 1922; "II, The significance of conjugation," *J. Exp. Zool.*, 39, 1924.

All the animals used in our studies were descendants from a single "wild" animal collected at New Haven. Standard beef extract was employed as the basic culture medium, and to this was added, as used, Colpidia raised in flasks of hay infusion. All the cultures directly compared were bred simultaneously under identical culture conditions.

The general method of conducting the pedigree lines may be briefly stated. From the original animal four lines were carried as the stem culture. Animals left over at the daily isolation were kept as stock, or allowed to conjugate or encyst. From time to time pairs of conjugants were isolated and their progeny established as "new" pedigree groups of cultures from the stem culture or its derivatives. Usually these groups comprised the progeny of two pairs of conjugants, though in a few cases the progeny of other pairs were also carried. In every instance the culture derived from a single exconjugant comprised four lines of animals and the average daily division rate of these four lines, again averaged for five-day periods, forms the basic data. At certain periods nearly 200 isolations were made daily, so that the investigation involved the manipulation of upward of 100,000 pedigree animals.

Passing over the question of the existence of a normal, inherent cycle in the life history of Spathidium, which we have already answered in the *negative* (cf. p. 230), we may summarize the data in regard to the effects of conjugation in cultures exhibiting a lowering of vitality under laboratory conditions.

A total of 118 exconjugants contributed to the experimental results. Of these, 18 died without dividing—a positive result—death immediately followed conjugation. However, the proportion of deaths is so small that it is negligible from the standpoint of this study. It is lower than the exigencies involved in such experimental work would naturally supply when the isolations and manipulations necessary are taken into account. Indeed,

the high viability of exconjugants is remarkable in comparison with that obtained by most investigators studying similar problems. In addition to the cases just mentioned, 6 other exconjugants need not be considered since they were lost by accident. Thus there remain 94 exconjugants which established cultures affording cogent data, of which 79 ran synchronously with their parent cultures for three or more five-day periods, and so supply definite evidence bearing on the question: Do the progeny of exconjugants characteristically exhibit a significantly different (higher or lower) rate of reproduction from that of their parent culture which has not so recently conjugated?

A study of the initial fifteen days of each of the 79 exconjugant cultures shows that 67 divided more rapidly and 12 less rapidly than their respective parents. The 15 other cultures, whose parental cultures died within the fifteen days, all actually divided more rapidly than their respective parents, so that they may with justice be added to the regular 67 plus cultures to give 82 cultures showing an initial increase in the division rate. However, it is immaterial whether the latter cases are included or not, so far as the conclusion which must be drawn is concerned. Obviously, the data overwhelmingly indicate that the rate of reproduction in the great majority of cases is accelerated after conjugation.

But the analysis thus far has involved merely contrasting the division rate of exconjugant and parent cultures on the basis of an increase or decrease of division rate, without considering the amount of difference. Clearly, before a conclusion is secure, it is necessary to take into account the chance variation which is inevitable in any experiment in animal breeding such as the present. The usual biometrical methods of analysis are inapplicable here because in each experiment the individual cultures must be treated separately, and judged in relation to the synchronous division rate of their respective parent cultures and also of their grandparental

cultures (if still surviving), as well as in relation to other cultures.

But the difficulties are more fancied than real, for by any reasonable criterion the number of cases in which the exconjugants exceeded the parent is far and away beyond the limits of any probable error that statistical methods may formulate. We have adopted a difference of five divisions in three periods, or an average of one third of a division per day, as a significant variation. Applying this criterion to the data gives 57 exconjugant cultures exceeding their respective parent cultures; 6 exconjugant cultures below their parent cultures, and 16 which are within this limit of error. A similar analysis in which seven divisions in three periods is taken as the significant variation gives 50 plus exconjugants, 5 minus exconjugants and 24 neutral. But our experience in handling the hundreds of pedigree lines during this experiment has convinced us that seven divisions in three periods is without doubt unreasonably large, and this is obvious when it is realized that this represents a change in the average rate of nearly one half a division per day. Nevertheless, even with this ultraconservative standard, the data give an unequivocal answer: conjugation in the great majority of cases directly induces an immediate acceleration of the reproductive activity.

A further analysis of the data, which need not be made at this time, fairly establishes for this pedigree race of Spathidium that conjugation usually is even more effective in accelerating reproduction when the exconjugants are derived from slowly dividing parents than when they are derived from rapidly dividing parents.

Turning now to the later effects of conjugation, we have 81 cultures which afford available data. Of these, 76 exconjugant cultures exceeded their parental cultures in the total number of divisions attained during their synchronous life, and 5 exconjugant cultures underwent a smaller number of divisions. Obviously this indicates that following conjugation the rate of division is accelerated.

But again, this analysis does not take into consideration the amount of difference in division rate between exconjugant and parental lines, and the difficulties involved here in determining a variation which is significant are still greater than in the comparison, just concluded, for the rates during a definite period of fifteen days. For now we are dealing with differences in duration: *e.g.,* some cultures ran synchronously for three periods and others for more than a dozen periods. Probably we can not do better than to take again the average variation of one third of a division per day as the criterion of a significant variation. Judged from this standard, 60 exconjugant cultures exceeded their parental cultures in division rate during their entire synchronous life, 3 exconjugant cultures were lower in division rate, and 18 cultures were within the limit of error of our standard.

Since conjugation is the sole variable involved in exconjugant and parental cultures, it is evident that conjugation directly induces not only an immediate acceleration of reproduction, but also an acceleration which persists at least as long as the life of the parental culture. And it is significant that the data from the first three periods and also from the entire synchronous life are practically identical, since this shows that the initial acceleration is maintained in essentially equal intensity to the end.

The question now to be considered is whether the progeny of exconjugants characteristically exhibit an increase or decrease in longevity in comparison with their parent culture which has not so recently conjugated. Among the 70 cultures which afford cogent data, 54 exconjugant cultures continued to live and reproduce after the death of their respective parent cultures, 10 exconjugant cultures died before their respective parent cultures, and 6 exconjugant cultures died essentially synchronously with their respective parent cultures. This analysis includes the cases where the exconjugant cultures when dividing rapidly suddenly died out in a day

without preliminary signs, because no criterion exists for positively determining that such deaths were "accidental." But since this fate befell more exconjugant than parent cultures, if these were omitted from the analysis the relative number of exconjugant cultures which survived their parent cultures obviously would be increased.

Thus the data presented provide a very definite answer. In the great majority of cases the exconjugant cultures outlived their parent cultures, and since the advent of conjugation is the sole variable involved, it is apparent that conjugation tends to increase the time duration of life as well as the rate of reproduction in Spathidium. Therefore, necessarily, the number of generations attained by the exconjugant cultures exceeds that of the parent after the origin of the former. In brief, under the conditions of these experiments, conjugation has a survival value in the life history of this organism.

This conclusion naturally leads to a consideration of the effect of conjugation on animals from cultures which are actually on the verge of extinction, in order to determine if conjugation has a direct and immediate survival value.

Four groups of cultures offer definite data. In each instance the progeny of the exconjugant survived while the parent culture died. And this evidence, direct as it is, has been amply corroborated in a very practical way by all the other work on Spathidium which for more than four years has been carried on at New Haven. Cultures about to die have been continued with exconjugants. It is clear, therefore, that conjugation, in the great majority of cases, has a direct survival value.

The relation of conjugation to the continuance of the life of the pedigree race from the parental to the F_5 generation under cultural conditions can be best illustrated by a specific example. From the parental culture at the thirtieth division the F_1 generation was derived; from

the F_1 at the 104th division, the F_2 generation was derived; from the F_2 generation at the 289th division the F_3 was derived; from the F_3 at the 150th division the F_4 was derived; and from the F_4 at the 80th division the F_5 was derived. The latter died at the 300th division.

Thus, from the parental culture to the death of the F_5 generation the series of pedigree cultures extended from November 5, 1920, to November 5, 1921, exactly a year, and the series could, without doubt, have been continued indefinitely by the same procedure. During the year the actual number of "linear" cell divisions from the parental culture through the F_5 generation of this series was 953. Since, under the conditions of the experiment, the parental culture died after 234 divisions on February 11, 1921, it is obvious that the life of the series, by five "conjugations" was prolonged approximately nine months, and exactly 719 divisions. Data may be similarly analyzed for the various generations of other groups, but it is unnecessary to do so here.

In brief, nothing can obscure the fact that under identical culture conditions "new" cultures, derived from conjugating pairs in an "old" culture, consistently survived the parent cultures. This, it appears, is a further definite demonstration of the survival value of conjugation.

The gradual "building up" of the division rate of a series through successive "conjugations" is also readily shown by the division rate of the same series of cultures which we have just been considering. The parental culture started with an initial rate of slightly above four divisions per day which was never again attained throughout its life. The F_1 generation attained but never exceeded the division rate of its parent. The F_2 generation never attained the maximum of its parental and grandparental cultures. The F_3 generation derived when its parent was dividing less than one and one half divisions per day and rapidly approaching extinction, exhibited a restoration of division energy which reached

three and one half divisions per day. However, this is below the initial maximum of the previous generations. The F_4 generation, derived when its parent was dividing approximately twice per day, immediately attained and maintained until discontinued a rate of nearly four divisions per day. This is almost as high as the maximum of the P and F_1 generations, identical with the initial maximum of the F_2 generation, and much higher than that ever reached by the F_3 generation. The F_5 generation, derived when its parent was dividing at an average rate of nearly four times per day, exhibited an initial rate of nearly four and one half divisions daily, the grand maximum for the entire series.

Granting that the series selected as an example is the most striking case the data afford, the fact remains that the other cultures, directly involved in the specific experiments to "build up" the division rate to a maximum equal or better than that ever exhibited before by this pedigree race of Spathidium, consistently show a similar cumulative effect of conjugation. Accordingly, all the data involved demonstrate that it is possible by successive "conjugations" progressively to increase, within limits, the maximum division rate.

Throughout all these experiments the animals employed were descendants from the one "wild" Spathidium isolated to start the stem culture, and therefore each generation (F_1 to F_5) in the series was the product of close inbreeding. As a matter of fact, the two animals which conjugated were in no instance more than eight divisions removed from a common ancestor. Since, as has been shown, conjugation effects an equal, if not greater, acceleration of the reproductive rate during the later experiments, it follows that continued close inbreeding, at least to the F_6 generation, does not significantly alter the typical physiological effect following conjugation at the F_1 generation. But in order to compare exconjugant cultures arising by such close inbreeding with similar cultures arising from the crossing of distantly

related animals, an attempt was made to cross animals which were separated by a large number of generations. The difficulties involved have thus far resulted in only one experiment out of over four hundred being satisfactorily carried through to completion. The animals mated were actually 982 divisions removed from a single common ancestral animal.

This culture and the necessary control cultures, involving 44 animals whose division rate was recorded daily for 25 days, showed no significant difference in rate of reproduction between the progeny of conjugants which were not more than eight divisions (about 48 hours) removed from a common ancestor and the progeny of the cross-conjugants, the members of which were removed 982 divisions (185 days) during which five "conjugations" were interpolated. Although no special weight should be given to this result, so far as it goes it is in accord with the data in regard to inbreeding from our other cultures.

In brief, all the data regarding the effects of conjugation derived from our study of Spathidium indicate a profound physiological stimulation of the metabolic activities of the cell which is expressed in reproduction.

As we know, endomixis is a periodic process coincident with the rhythms during the free life of Paramecium aurelia, P. caudatum and P. polycaryum.[13] In Paramecium calkinsi, endomixis is absent though rhythms occur; in Didinium and Uroleptus, according to Calkins, and in Spathidium, according to Moore, it occurs during encystment. In Paramecium calkinsi it obviously is not a necessary phenomenon, and the same conclusion has been drawn from data on Spathidium. But, when it does occur, has it a significant physiological effect?

In Paramecium aurelia and P. caudatum a higher division rate certainly follows endomixis, since recovery from a rhythm is coincident with endomixis. However, since

[13] Woodruff and Spencer, "Paramecium polycaryum, sp. nov.," Proc. Soc. Exp. Biol. & Med., 20, 1923.

in other Infusoria (*e.g.*, Paramecium calkinsi) recovery from rhythms regularly occurs in the entire absence of endomixis, it is impossible to definitely determine a causal relation between endomixis and recovery from rhythms in Paramecium aurelia and P. caudatum.

In pedigree cultures of Didinium, Calkins found that periodically all the animals encysted for purposes of reorganization, or endomixis, and accordingly he interpreted the process as possessing a survival value for the race. Mast in his studies of the same species did not find periodic encystment and stated it to be improbable that encystment is necessary for the continued existence of Didinium or that it has any appreciable effect on the death rate or fission rate.[14] The data from Uroleptus, however, certainly indicate, as Calkins says, that after encystation, during which endomixis takes place, the animals possess an initial vigor similar to that of an exconjugant. Furthermore, split conjugants, that is, animals separated or cut apart immediately after contact and therefore long before the definitive nuclear changes of conjugation have occurred, undergo an endomictic process which results in rejuvenescence exactly similar to that following conjugation. Apparently the mere contact of two animals initiates the nuclear changes for conjugation, and when conjugation is thwarted the cells meet the emergency by an endomictic process which typically only takes place in the species during encystment.

Weighing all the available facts the conclusion seems justified that when degeneration is in progress endomixis has a rejuvenating effect, and we thus reach the general result that *both* conjugation and endomixis have a similar effect under similar conditions—both meet the emergency of physiological degeneration *induced by environmental conditions* which are not ideal. The fact that Paramecium aurelia and P. caudatum are so widely distributed and are able to exist under such diverse surroundings

[14] Calkins, "Didinium nasutum, I, The life history," *J. Exp. Zool.*, 19, 1915; Mast, "Conjugation and encystment in Didinium nasutum with special reference to their significance," *J. Exp. Zool.*, 23, 1917.

can reasonably be interpreted as due to their having instituted endomixis in the free-living state. And furthermore, it is possible, we believe, to explain in part, at least, the fact that Jennings's remarkably intensive study of conjugation in Paramecium failed to reveal acceleration of cell division: endomixis, which was then unknown, tended to maintain his control lines at a high level. At all events, crucial experiments on the physiological effects of conjugation are only possible in forms in which endomoxis is not a complicating factor.

If the conclusion is secure that both endomixis and conjugation rejuvenate, it is clear that synkaryon formation is not the essential factor, since this does not occur during endomixis. The cytological evidence, which is solely from Paramecium, indicates that in endomixis the first two of the three 'pre-synkaryon' divisions occur, one of which during conjugation is usually interpreted as a reduction division, though recently Dehorne has claimed that it is the third division. Since, however, the nuclear changes in conjugation and endomixis are apparently fundamentally the same except for the gamete-forming division, endomixis in Paramecium offers strong physiological evidence against the interpretation of either of the first two divisions in conjugation as effecting chromosome reduction, because, if one of the divisions in endomixis is reductional, then there must be somehow, somewhere, a compensating mechanism, for which there is no cytological evidence and against which there is much physiological evidence.

Erdmann from studies on size variation after endomixis in Paramecium has found that variations arise, some of which are heritable.[15] These, however, apparently fall in the same category as the variations which Jennings and his colleagues have demonstrated in pure lines of various Rhizopods, and which are almost surely independent of segregation and recombination phe-

[15] Erdmann, "Endomixis and size variations in pure bred lines of Paramecium aurelia." *Arch. f. Entw.-mech.*, 46, 1920.

nomena as understood in the metazoa. In short, the physiological effect, so far as rejuvenescence is concerned, common to both endomixis and conjugation must be sought in intracellular changes independent of those generally interpreted as following from genetic factors.

The universal method of reproduction in the Protozoa, as, of course, in final analysis in all organisms, is cell division. But while in higher animals reproduction is usually associated with fertilization, in the Protozoa there is clearly presented the essential separateness of these processes since when fertilization occurs it merely punctuates a longer or shorter series of independent organisms which are the products of fission. Fertilization, however, is apparently neither of such universal nor frequent occurrence in the Protozoa as was formerly believed to be the case. Following the early observations of Dallinger and Drysdale on the life history of Monads and the discovery of fertilization in the Infusoria, it was confidently predicted that only further research was necessary to reveal it in all forms. And Schaudinn's beautifully conceived and executed studies on the life histories of a series of Protozoa from Endamoebae to malarial organisms seemed to justify the idea. But recent and intensive work on the Sarcodina and the Mastigophora has signally failed in many cases of the former and most cases of the latter to supply the critical evidence of synkaryon formation necessary to pronounce fertilization a fact, and it certainly must be left an open question whether it is not the exception, rather than the rule, in these classes. At all events, and this is the point of immediate interest, asexual reproduction is obviously adequate in the Protozoa, as it apparently is in certain, at least, of the Protophyta, to carry on the stream of life uninterrupted. It is yet premature to attempt to trace the evolution of sexuality in the Protozoa, but if one may be permitted to hazard a guess, it will prove to be of diphyletic origin and from some nuclear reorganization processes endomictic in nature.

Enough has been said to recall to mind the rapidly changing picture which the so-called dynamic aspect of the problem of fertilization has presented since the pioneer work on conjugation in the Protozoa. Its investigation has been fostered very largely by the belief that the key to the fertilization of higher forms must be sought in the conjugation of unicellular organisms; and the Infusoria, it is hoped, have not so far proved disappointing, when we bear in mind that they have had as long an evolutionary history as man and exhibit in their *multum in parvo* plan of structure a complexity as real as it is different.

Reprinted from the Proceedings of the National Academy of Sciences,
Vol. 11, No. 7. July, 1925.

A HISTOLOGICAL STUDY OF STERILITY IN THE ALBINO RAT DUE TO A DIETARY DEFICIENCY

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Read before the Academy April 27, 1925

In the winter of 1921–22, at the request of Dr. Osborne and Dr. Mendel, a thorough study was begun to determine the histological condition of the reproductive organs of rats fed artificial diets of known composition, adequate for growth but not for reproduction.

So far as we can find C. B. Paul[1] was the first to intimate that fertility is affected by diet. He states that "an excessive meat diet interferes with the development of the male reproductive organs" and that "the defective development is most pronounced in the seminal vesicles and prostate

gland." Hewer,[2] after excessive thymus feeding of rats, described a pronounced histological degeneration of the testes but normal conditions of the epididymis.

Funk and Douglas[3] next called attention to the atrophied testes and ovaries of pigeons suffering from beri-beri, but gave no evidence to show whether this condition was directly due to a deficient diet, or to the diseased condition of the birds.

Osborne and Mendel[4] noticed that although animals fed on their artificial diets "had grown vigorously to adult size they were, with very few exceptions, sterile." An examination of some of these animals, made by Allen,[5] showed "total degeneration of all the germ cells" in the testes.

Dutcher and Wilkins[6] found that "the testes of White Leghorn cockerels did not develop when the diet consisted of polished rice," but that "when supplemented with small amounts of green alfalfa atrophy of the testes did not occur."

After our own studies were begun Evans and Bishop[7] published a study of the effect of diet on the oestrous cycle. Meyerstein[8] reported histological differences between both the testes and ovaries of rats fed on diets low in the water-soluble vitamine compared with the corresponding organs of those receiving a larger quantity of this vitamine. Eckstein[9] found advanced degeneration of the testes but no evidence of abnormality of function in the ovaries of rats after feeding on diets poor in fat, vitamine A and phosphorus.

Evans and Bishop,[10] and other workers, have since shed much light on the female disorder and its relation to the reproductive vitamine. After learning that Evans and Bishop were studying the effect of diet on the female rat, the writer directed his attention to the male, with the results given in this paper.

Preliminary Work.—In the preliminary part of this work,[11] involving breeding tests, autopsies and histological studies of 20 male and 40 female rats reared under dietary regimes of several types, the following results were obtained: (1) No reproduction was secured in rats reared for 125 days or more on the standard casein diet (casein 18%, starch 54%, lard 15%, butter fat 9%, salt mixture[12] 4%), together with tablets of dried yeast, alfalfa, clover or dandelion, fed daily to supply a sufficient amount of water-soluble vitamine for normal growth. (2) A histological study of the ovaries showed a normal process of maturation and ovulation, followed by what seemed to be normal uterine implantation, although the embryos were aborted or resorbed in the late stages of gestation. (3) After prolonged feeding, the testes of the males were usually small and watery, and showed a degenerate histological condition. (4) Occasional reproduction was secured on a similar casein diet, containing egg yolk (casein 28%, starch 39%, lard 15%, butter fat 9%, salts 4%, egg yolk

5%, and tablets of 0.2 gm. of yeast fed daily). Excellent growth was obtained on both diets.

Experimental.—The purpose was to determine the period at which the degeneration becomes evident, the duration of the process, and the detailed histological and cytological changes involved.

The diet adopted as the basic ration in these experiments was casein 18%, starch 54%, lard 15%, butter fat 9%, salt mixture 4%, plus yeast tablets 0.4 gm. fed daily, which is successful in so far as the growth and vigor of the rats is concerned, although animals reared on this diet from the time of weaning never produce young. In the work done on recovery from sterility, this diet was supplemented with 40 gm. of fresh green lettuce fed daily, to supply the vitamine E. Of the 130 male rats used, 95 had one testis removed by operation (unilateral castration) before or after maintenance for various periods on the basic ration. After different periods of time the other testis was removed. Both testes of each rat were then studied histologically to determine the degree of degeneration after the different periods of dietary treatment, as well as the changes occurring in the same rat in the interval between these periods.

Results. (*a*) *Histology of the Testicular Degeneration.*—For convenience the degenerative process has been divided into five stages, as follows.

Stage 1. The mature sperm lose their individuality, clump together to form irregular masses or blotches of degenerate chromatin material, and are eventually passed off through the epididymis. Other stages of the spermatogenic process are still normal.

Stage 2. The sperm have disappeared in most of the tubules and the spermatids show a "beaded" appearance due to rupture of the nucleus and deposition of chromatin upon the nuclear wall. The cell walls of the spermatids seem to disappear towards the end of this stage, and the cytoplasm of the germinal epithelium segregates and rounds off in certain portions of the tubule preparatory to the formation of the giant cells of the next stage. The spermatocytes and spermatogonia appear normal. This stage is of very short duration, as indicated by its rather rare occurrence in the large number of testes studied.

Stage 3. The "giant cell" stage resulting from the segregation of rounded masses of cytoplasm in which are included from 2 to 40 degenerating spermatid nuclei. These multinuclear giant cells become pycnotic and eventually liquefy, possibly causing the rather watery condition of testes in this and later stages of degeneration. Many degenerate spermatids do not enter into the formation of the giant cells, but become detached and accumulate in large masses which are then passed to the epididymis. The spermatocytes usually show irregular clumping of the chromatin material on the nuclear wall. The cytoplasm of the epithelium becomes fibrous and vacuolated due to the sloughing off or dissolution of degenerate germ

cells and giant cells of all sizes. This stage is of relatively short duration.

Stage 4. A few giant cells in different stages of dissolution are usually present, along with spermatocytes and spermatogonia in stages of necrosis, although the latter are usually sloughed off from the germinal epithelium before degeneration is very marked.

Stage 5. Shows the histological condition of a cryptorchid testis. The germinal epithelium is reduced to a syncytium of Sertoli cells in a fibrous stringy layer of cytoplasm, which forms a thin layer near the periphery of the tubule or almost fills the inter-tubular space. The Sertoli cells and the interstitial cells show no degeneration, even in the most extreme cases. There is, however, a very marked shrinkage in the size of the tubules.

In advanced conditions the epithelium lining the ducts of the epididymis show changes indicating fatty degeneration.

(*b*) *Rats Reared on the Basic Ration from Weaning.*—After similar periods on the diet these rats, especially those from different litters, show wide variations in their testicular condition. The various types of degeneration reached at the different stages of dietary treatment are shown in Table 1. The animals were assigned to the several stages according to the condition of the majority of the tubules. None appear in the table under Stage 2 although in many cases some of the tubules were in the condition belonging to this stage. When sexually mature animals are fed on this diet this stage is more apparent, due to the slower degeneration of the testes.

TABLE 1

DEGREE OF TESTICULAR DEGENERATION IN RATS FED FROM WEANING ON THE BASIC RATION FOR 12 TO 150 DAYS

PERIOD OF DIETARY TREATMENT DAYS	NO. OF TESTES STUDIED	NO. OF NORMAL TESTES	NUMBER OF TESTES IN				
			STAGE 1	STAGE 2	STAGE 3	STAGE 4	STAGE 5
12– 40	4	4	.	.	.	.	.
50– 55	16	5	9	.	2	.	.
60– 65	15	6	8	.	.	1	.
70– 75	11	4	.	.	1	6	.
80– 90	14	1	4	.	1	7	1
100–150	12	.	.	.	.	4	8

The above data show that degeneration begins between 50 and 60 days after weaning, and that after 100 days on the diet degeneration is practically complete.

Table 2, based on Donaldson's figures for the normal, shows the relative weights of the body and testes.

TABLE 2

SHOWS BODY WEIGHT AND TESTIS WEIGHT (COMPUTED AS PERCENTAGE NORMAL BY WEIGHT) OF RATS REARED ON BASIC RATION AFTER WEANING

PERIOD OF DIETARY TREATMENT DAYS	NUMBER OF RATS USED	RATS % NORMAL BY WEIGHT (AVERAGE)	NUMBER OF TESTES WEIGHED	NORMAL WEIGHT OF TESTES (AVERAGE)	ACTUAL WEIGHT OF TESTES (AVERAGE)	TESTES % NORMAL BY WEIGHT
12– 40	4	148	..	....	....	....
50– 55	16	126	8	1.96 g.	2.12 g.	108
60– 65	15	122	14	1.99 g.	1.97 g.	99
70– 75	13	118	9	2.13 g.	1.61 g.	75
80– 95	16	118	14	2.25 g.	1.62 g.	72
100–150	12	128	5	2.67 g.	1.28 g.	44

(*c*) *Rats Fed on the Basic Ration after Attainment of Sexual Maturity.*—Sexually mature normal animals, varying in age from 60 to 105 days, were kept on the diet for 25, 50, 75 and 100 day periods before histological study of the testis. Table 3 shows that testicular degeneration is slower in asserting itself under such conditions, the critical period usually lying between 75 and 100 days.

TABLE 3

SHOWS THE DEGREE OF TESTICULAR DEGENERATION IN SEXUALLY MATURE RATS MAINTAINED ON THE BASIC RATION

PERIOD OF DIETARY TREATMENT DAYS	NO. OF TESTES STUDIED	NUMBER OF TESTES IN					
		NORMAL	STAGE 1	STAGE 2	STAGE 3	STAGE 4	STAGE 5
25	5	5	.	.	.	.	.
50	12	11	1	.	.	.	.
75	10	7	1	.	1	1	.
100	11	1	3	2	.	.	5

(*d*) *Prevention of the Testicuar Disorder.*—Rats held on the basic ration until the testicular degeneration had begun, as determined by a study of one testis removed at operation, and then placed on the stock food diet (dog-biscuit plus carrots) for periods up to 75 days, show no improvement in the histological condition of the other testis, degeneration continuing as if no change had been made in the diet. If, after removal of one testis, the rats are given 40 grams of fresh lettuce daily, but kept on the basic ration for periods up to 150 days after operation, the second testis may show evidences of "partial prevention" provided some normal or practically normal seminiferous tubules were present at the beginning of lettuce feeding. Lettuce prevents the degeneration of such tubules, while those already in early and later stages of the disorder continue to stages of extreme degeneration. Of 36 rats, 20 in this series showed varying degrees of "partial prevention," but gave no indication that, once degeneration has set in, they can return to normal.

Lettuce fed from the beginning of the experiment prevents the testicular changes which would otherwise occur.

* The writer wishes to express his thanks and indebtedness to Dr. R. G. Harrison under whose direction the histological studies of this problem were carried out, at the Osborn Zoölogical Laboratory; and to Dr. T. B. Osborne and Dr. L. B. Mendel for their encouragement and coöperation in connection with the dietary work, the expenses of which were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institute of Washington, D. C.

[1] Paul, C. B., *J. Physiol.*, 1906, **34**, p. XIV.

[2] Hewer, E. E., *J. Physiol.*, 1914, **47**, 479.

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TRANSACTIONS OF
THE CONNECTICUT ACADEMY
OF ARTS AND SCIENCES

VOLUME 27] JULY 1925 [PAGES 51-248

Arachnida from Panama

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NEW HAVEN, CONNECTICUT
PUBLISHED BY THE
CONNECTICUT ACADEMY OF ARTS AND SCIENCES
AND TO BE OBTAINED ALSO FROM THE
YALE UNIVERSITY PRESS

THE TUTTLE, MOREHOUSE & TAYLOR COMPANY, NEW HAVEN, CONN.

ARACHNIDA FROM PANAMA

INTRODUCTION

The study of the Arachnid fauna of the West Indies and of the shores of the Caribbean Sea has a peculiar interest in view of the geological past of the Antilles and because of the methods of distribution in this group of Invertebrates. The dispersion of individuals is accomplished by different means in different Orders of the Class and often by different means in different Families of the same Order. Dispersion by flight is possible only in spiders, by means of gossamer threads, and in mites parasitic on birds or insects. In the latter case the distribution of the species is evidently limited by the habits of the hosts; in the former by numerous factors as yet little known, such as the season of the year, air currents, humidity, temperature, climate and food. To a not inconsiderable extent, perhaps, also by the longevity of the species. We know something of the dispersion of spiders in the temperate zone where only those species which emerge from the egg in autumn produce gossamer threads. For this reason closely related genera sometimes show a decided difference. Thus in the United States *Argiope* winters in the state of eggs while *Epeira* develops earlier and takes part in gossamer flight. Definite information on ballooning exists only in the case of eight genera, and of these only one, *Atypus,* belongs to the Sub-Class Mygalomorphæ. Of ballooning in the tropics we know nothing. We do not even know whether it occurs there at all. Nor do we know the distance to which spiderlings may be carried by the wind, nor the height from the ground to which they may ascend in rising currents of air, both factors of the greatest importance in the distribution of spiders of an archipelago.

Floating objects and human trade may be considered as an important factor in the dispersion of Arachnida, applicable probably to all Orders, but only to such species as frequent human habitations or hide in crevices or under the bark of wood, in fruit, among living plants and other objects of export. We know about two dozen species of spiders and a few scorpions which are cosmopolitan in their distribution and which may have

been introduced in some places through the agency of man or oceanic currents. But trade involves usually great distances and considerable change of climatic conditions. Arachnida do not depend upon a certain species of insect for their food, but in new surroundings and new climate they may not find any food at a time of the year when they are particularly in need of it, or they may not be able to stand the cold or the lack of it, or they may encounter unaccustomed enemies, or conditions unfavorable for nest making or some other factor adverse to their existence. In many cases too, the lack of an individual of the opposite sex at the time of mating may prevent the establishment of a species in a new country even when the other conditions are favorable. While it is easy to see how all these factors work against the importation of southern species into northern countries, it is more difficult to understand why northern species are also restricted in their distribution. Yet we know that many species occur only in the North. What stands in the way of their gradual distribution southward?

Probably the most common method of dispersion in Arachnida is that of walking a sufficient distance away from the place of their birth to prevent cannibalism and to assure a sufficient supply of food. But this method is good only on land. Although we know nothing of the methods of dispersion in cave-dwelling Arachnida, it seems likely that it is the only possible method in these species.

A fascinating picture unrolls itself before the mind of the investigator who could compass at once in all its phases the arachnid fauna of the West Indies and the shores of the Caribbean. He could trace the geographical distribution backward to the centers of dispersion; he could identify the species which have sprung from the original stock after its geographical isolation; he could put his finger on the environmental factors which were responsible for the appearance of the new characters; he could comprehend the adaptive modification of instinct; he could envisage the evolutionary tendencies and bring the problem of evolution itself nearer our understanding. The hope some day to be able to contribute materially to our advance in this direction has ever been present during the many years of my studies. Unfortunately it soon became apparent that even our knowledge of the species inhabiting those regions is quite inadequate. No comprehensive

lists, no data of localities or of time of capture exist, and the descriptions of new species even by the most distinguished arachnologists are often too inadequate for identification, let alone for the study of local variations. Nor have been any biological observations made in the field, none at least of a comparative nature. It is therefore absolutely indispensable to begin at the beginning by a patient study of collections, compilation of lists and careful description of new and of inadequately described species. Naturally, this kind of work need not and cannot follow a prearranged system. It depends upon collections available, and may require additional investigations in its course.

The present paper, dealing mostly with spiders from Panama, is chiefly based on the study of a collection made in the spring of 1924 by a student in the Zoological Department of Yale University, Mr. J. M. Valentine, who went there for other purposes, but who was enabled to remain longer and to collect arachnida through a grant of $100.00, made at my request, from the Elizabeth Thompson Science Fund. It gives me great pleasure to express my gratitude for this help to the Trustees of the Fund, as the collection has proved to be rich in new species. A few smaller collections have also been examined during the preparation of this paper. These were made at different times by Dr. B. H. Buxton, formerly Professor of Pathology in the Cornell Medical School, Mr. W. J. Baerg, Entomologist in the Agricultural Experiment Station of the University of Arkansas, and by Miss Elizabeth Deichmann. The latter collection was placed at my disposal by Dr. C. R. Shoemaker of the U. S. National Museum. To all these I express my best thanks for their courtesy and interest shown in my work.

While the smaller collections were all made in the Canal Zone and in the vicinity of Panama City, the localities in which Mr. Valentine was collecting require special attention. The localities visited by him are all east of the Canal Zone and either on the Pacific coast or on the Pacific slope of the mountain range. Mr. L. Griscom, with whom Mr. Valentine was traveling, describes the route and the collecting ground in an article published in No. 4, Vol. XXIV of Natural History for July-August 1924 on pages 509-519. *Remedios* and *Sona* are almost on sea-level with mangrove swamps in their vicinity. *San Lorenzo* is a river the swampy shores of which are covered with tropical forest. *Cerro*

Iglesia is in a tropical forest about 700 to 1,000 feet above sea level. *Bocas del Monte* represents an arid zone about 3,500 feet above sea level. *Cerro Flores* (sometimes referred to as Cerro Santiago on maps) is a mountain on the slope of which in a sub-tropical forest collecting was done at an elevation of from 3,700 feet to about 5,000 feet.

Most of our knowledge of the spider fauna of Panama was based upon the work of the two Cambridges forming part of their two splendid volumes on Arachnida Araneidea in the Biologia Centrali Americana. Whatever has been added since is of a desultory and chance character. It is perhaps too early to form a definite opinion on the arachnid fauna of Panama, but the indication is unmistakable that this fauna has more relationship to the Central American than to the South American fauna. I reserve a more detailed account of this relationship for future time, when I shall bring my study of the fauna of other Central American countries and of the West Indian Islands nearer completion. For the present this paper, the first of a series which is in preparation, must remain a strictly taxonomic one.

TERMINOLOGY AND MEASUREMENTS

The terminology employed in this paper is practically the same as in my paper which is to be published in the Annals of the Entomological Society of America except for a little improvement in the notation of spines on legs. To understand it I must recall to the mind of the reader that I use the terms *dorsal* and *ventral* in their morpological sense as determined by the plane of symmetry. *Right* and *left* are reserved for the body only. For all appendages the terms *pro* and *retro* are used to designate the two surfaces forming right angles with the dorsal and ventral surfaces. The *prolateral* surface is always the one which is nearest the anterior end or head of the animal. In a front leg the prolateral surface is therefore the inner or right side in the left leg and the inner or left side in the right leg. In the hind leg it is the outside or left side of the left leg and the outside or right side of the right leg. The *retrolateral* surface is always the one farthest from the head and nearest the posterior end of the spider. It is therefore the outside of the front legs and inside of the hind legs. The term *promargin* is applied to the inner margin of the cheliceræ of Theraphosid spiders and to the upper or outer margin of the cheliceræ of true spiders, while the term *retromargin* is applied to the outer margin of the cheliceræ of Theraphosid spiders and to the lower or inner margin of the cheliceræ of true spiders. The planes are determined by imaginary straight lines connecting the articulation points of the apical and basal end of the same segment. In the tibia there is a single middorsal articulation with the patella. It is necessary therefore to draw the imaginary line from the femoro-patellar to the tibio-metatarsal articulation. Extensive study has persuaded the writer that, with rare exceptions, no more than two rows of spines may be present on any of the four planes. A pair of spines is always represented by the figure 2, whether the spines are in a symmetrical position on the right and left of the median line or one spine in advance of the other. A single spine situated in the middorsal, midventral or midlateral line is similarly represented by the figure 1. Thus dorsal 2-2-2 means three pairs of spines, dorsal 1-1-2 means that the two apical spines form a pair. If the absence of a spine or of a pair of spines is quite certain from a comparison with the spines of another pair

of legs such absence is represented by the cipher 0 (I have in mind of course actual absence, not loss due to injury). Thus dorsal 1-0-1 means that there are only two spines in the middorsal line, separated by considerable space in which on a leg of another pair there is a spine. If only one spine of a pair has been lost, or if a single spine is situated not in a median line, then such a spine is represented by the numeral 1 with addition of a letter p, r, d or v. These letters indicate the plane nearer to which the single spine is situated. Thus dorsal 2-1r-2 means that the single spine is in line with the preceding and following which are closest to the retro-side of the leg. Similarly prolateral 1d-1v-1d-1v means that the four spines are not in one line but that the first and third are nearer the dorsal surface than the second and fourth. Though this system seems to be somewhat complicated at the first glance, it is not only more exact than either of the two systems generally in use, but once learned is no more difficult than the others. Mention should be made of cases in which either the legs are too thin or the spines too numerous to allow of a careful definition of the planes. In such cases no notation may be quite safe. The case of so-called vorticella of spines, as for example at the end of metatarsi in many jumping spiders, is only an additional proof of the inadequacy of the old systems of notation. Whenever there are six spines at the end of the metatarsus a pair of these is always ventral, a pair prolateral and a pair retrolateral. If there are only five spines the new notation shows at once which of the spines is wanting. It will be also found that in some cases there is a bristle in place of a spine which is present in the identical place on another leg. In all such cases the word *bristle* is added to the numeral.

All measurements are in millimeters and their fractions and, with the exception of very large specimens, are made with the aid of a micrometer eyepiece under a binocular microscope. It is essential that the entire segment should be in focus as otherwise wrong figures will result. For appendages the measurements are made from articulation point to articulation point. In the case of the eyes care must be taken to measure the *diameter of the cornea,* not of the better visible pupil which is often much smaller. All our measurements represent the diameter of the cornea or, in case of oval eyes, the *longer axis.* The measurement of the length or width of the eyegroup or of the quadrangle of median

eyes invariably includes the eyes, but not the curvature of the eyerow. Similarly, the length, width and height of the carapace, unless otherwise specified, does not include the curvature, but represents an airline. The ratios of eyes are naturally nothing but ratios. They have been purposely left without any change whatsoever, without any attempt to refer the figures to some definite organ. These ratios represent simply the number of divisions of the scale of the ocular micrometer. It is difficult to choose an organ sufficiently permanent in its relative size to be used as a unit for comparison, and the retention of the original figures, which are interchangeable, reveals relationships which are otherwise lost.

ORDER SCORPIONES

Of this Order only a few specimens from Panama came to my observation. One male and one female of *Opisthacanthus elatus* Gervais from Culebra are the only representatives of the Family Scorpionidæ. The Family Buthidæ is represented by one male *Tityus cambridgei* Pocock from Culebra, one male *Centrurus gracilis* Latreille from Bocas del Toro, and five females and two immature specimens of *Centrurus margaritatus* Gervais (locality not given).

ORDER PEDIPALPI

Of this Order only two species from Panama came to my notice. Five specimens of *Tarantula palmata* Herbst (locality not given) and one specimen of *Tarantula fuscimana* C. Koch from Bocas del Toro and six specimens from the Wilcox camp on the San Lorenzo River.

ORDER OPILIONES

Our ignorance of this Order is truly appalling. Only four species have been so far recorded from Panama, all belonging to the Sub-Order Laniatores. Of these *Flirtea picta* (Perty) is known also from Brazil, and *Euerginus subserialis* (Cambridge) from Costa Rica and Guatemala, while *Cosmetus flavopictus* Simon and *Hernandarioides plana* Cambridge from Panama only. To these must now be added the following species as occurring in Panama:

Family Cosmetidæ

Eucynorta quadripustulata Simon—Several specimens from Colon. (The type was from Colombia without locality.)

Eucynortula lata (Banks)—Three specimens from Panama without locality. Occurs also in Costa Rica and in Nicaragua (a specimen in the collection of the Dept. of Entomology, Washington, D. C.).

Poecilema limbatum Kollar—one specimen from Cerro Flores. (Originally described from Brazil.)

Family Phalangodidæ

Metapachylus gracilis Cambridge, one specimen from Cerro Flores. (Previously known from Mexico.)

Stygnomma rufum sp. nov., one specimen from Cerro Flores. (I have also three specimens of this species in my collection from Limon, Costa Rica. I have also one male and one immature specimen of another species of the same genus, *Stygnomma armatum* sp. nov., from Siquerres, Costa Rica. Since our knowledge of the genus has been hitherto limited to a single species, *Stygnomma fuhrmanni* Roewer, represented by two males from the plateau of Camelia in Colombia, I give here a description of both new species.)

Metapachylus gracilis Cambridge.

Total length 4.5 mm. Legs, I—4.85 mm., II—8.00 mm., III—6.63 mm., IV—9.18 mm., Palp—2.55 mm.

Dorsal scutum divided into five areas. Carapace much narrower than scutum, with parallel sides and squarely truncated front with each angle produced into a spine. Lateral margin of scutum with tubercles. First area with scattered setigerous tubercles, second with two transverse rows, third, fourth and fifth areas with a single row of setigerous tubercles. Eyetubercle high, with a long, curved horn directed forward and carrying a short seta at the end. Chelicerae small. Pedipalpi powerful, their trochanter with one setigerous tubercle below, femur with two near base below and one prolateral near apex, patella with a single one prolateral, tibia and terminal joint with two pairs each below. At the end of the terminal joint a powerful, smooth claw. Both fingers of chelicerae toothed, the movable finger from middle on, the immobile one from its base on. These teeth are rounded at the end. Setigerous tubercles are present on all legs on every segment except first to third metatarsi and all four tarsi. The third trochanter is globular. Subdivision of the tarsi as follows: first tarsus—3 joints, second tarsus—4 + 3 or seven in all, third tarsus—3 + 2 or five in all, fourth tarsus—6 joints; all joints free. Pseudonychium on third and fourth tarsus wanting. Claws all smooth. Scopulae on legs wanting. Spiracles not visible. Color in alcohol brown. Palpi and trochanters of all legs yellow. Dorsal scutum with a black submarginal line. Carapace mottled with black, areas with indistinct black sprinkling which assumes the aspect of a black, transverse line on the free abdominal tergites. Habitat: Cerro Flores, Panama.

Although Roewer has placed the genus *Metapachylus* in the

Sub-Family Pachylinæ of the Family Gonyleptidæ he adds in a footnote on page 449 of his great work "Die Weberknechte der Erde": "Cambridge's Diagnose u. Figur nach scheint das Tier zu den Phalangodidæ zu gehören, wenn nicht in der Diagnose das Psd des 3. u. 4. Ta besonders hervorgehoben wäre. Bei der Unsicherheit der Angaben in Cambridge's Diagnose muss dieses Tier bis zur Nachprüfung der Type als unsichere Art u. Gattung bezeichnet werden." I have had no opportunity to examine the type, but the specimen in my collection agrees in every detail with the figures of the type made by Cambridge. It seems to me that Roewer has misunderstood Cambridge's statement "tarsal claws of iii and iv, with an inter-basal process" and interpreted it as meaning that there is a pseudonychium on these joints. In my specimen there is also a small process, but it has the appearance of a little cone in no way comparable to a pseudonychium of other Opilionids. I do not hesitate to place the species in Roewer's Sub-Family Erecananinæ of the Family Phalangodidæ.

Stygnomma rufum sp. nov.

Total length without cheliceræ 5.0 mm. Legs, including trochanters, I—12.3 mm., II—16.5 mm., III—12.0 mm., IV—15.5 mm., Pedipalpi, including coxa, 10.0 mm.

Dorsal scutum with 5 areas, none of which has a longitudinal sulcus. Eyes far apart, each on a separate small tubercle. Between and slightly behind the eyes a tubercle with a central larger cone-shaped spine and an irregular circle of smaller tubercles. Transverse rows of setigerous tubercles on every area and on all free abdominal tergites. Spiracles well visible. Entire ventral surface including coxae covered with setigerous tubercles. First joint of cheliceræ 3.0 mm. long, with 3 pointed tubercles above and a right and left lateral row below together with several irregularly distributed tubercles. Chelæ much heavier than first joint, moveable finger considerably shorter than hand which is covered only with small setæ. Pedipalpi powerful and long; their coxa curved and armed dorsally with a row of teeth of which the distal one is strongly curved forward, ventrally with a retrorow of 4 setigerous tubercles and a prorow of several small tubercles. Trochanter of pedipalp short, with a peduncle and 2 dorsal teeth and 3 ventral setigerous tubercles. Femur curved, gradually increasing in width toward apical end, with a ventral

row of setigerous teeth. Patella with peduncle which is ⅓ the full length of the segment, with 2 setigerous teeth below. Tibia with 2 pairs of heavy setigerous tubercles below. Terminal joint with 2 pairs of similar tubercles and a heavy, smooth terminal claw. All trochanters and femora of legs with setigerous tubercles, also fourth patella and tibia. Trochanters of third pair heaviest, globular, three times as heavy as femur; those of fourth pair subglobular, smaller. Trochanters of first and second pair with straight ventral and convex dorsal surface, elongate. Number of tarsal joints, I—5 + 2 or seven in all, II—7 + 3 or ten in all, III—3 + 3 or six in all and IV—4 + 3 or seven in all. First and second pair of tarsi with a single claw, third and fourth with a pair of claws. Scopulæ wanting. Claws smooth. Color in alcohol rufous throughout except fingers of chelicerae, which are darker. Habitat: Cerro Flores, Panama and Limon, Costa Rica.

Stygnomma armatum sp. nov. (Figure 1).

Male. Total length of body 6.0 mm. Legs, I—12.92 mm., II—18.36 mm., III—12.58 mm., IV—15.90 mm. Palpi 11.22 mm. number of tarsal joints, I—7 (= 5 + 2), II—13 (= 10 + 3), III—6 (= 3 + 3), IV—7 (= 4 + 3). Chelicerae very large,

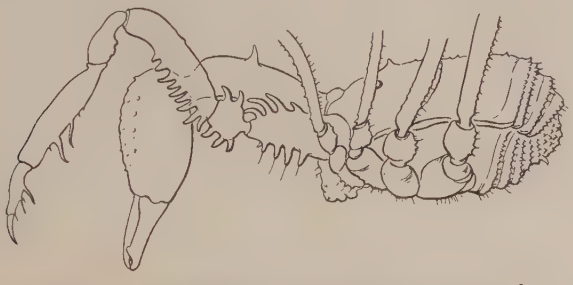


FIGURE 1, **Stygnomma armatum** sp. nov., male.

long and heavy, with a single dorsal tubercle on first segment. Length of first segment—3.57 mm., of hand with finger—5.185 mm. Pedipalpi armed in same way as in S. rufum, except that the spines are longer and stronger curved forward. Color in alcohol a mottled grey except first joint of chelicerae, and pedipalpi, which are white. Legs dark below, but with white ends in all segments except metatarsi and tarsi. Cephalic line of scutum, separating head, black. Habitat: Siquerres, Costa Rica.

ORDER ARANEÆ

In the following list of spiders recorded as occurring in Panama an asterisk denotes that the species has not been previously reported from that country, while all new species are in black face type.

SUB-ORDER MYGALOMORPHÆ

Family Theraphosidæ

Avicularia glauca Simon.

Eurypelma emilia (White).

Eurypelma panamense Simon.

**Eurypelma sabulosum* F. Cambridge, 1 female from Culebra. Original record from Guatemala.

**Eurypelma vagans* Ausserer, 1 female from Culebra, 4 very young specimens probably belonging to this species without definite locality.

**Hapalopus pentaloris* (Simon), 3 immature specimens without definite locality. Known from Guatemala, Costa Rica and Mexico.

**Ischnocolus* sp.?, 2 immature specimens from Culebra, 1 immature specimen from Cerro Flores, found under a log in the forest. All specimens much alike and with the characters of the genus as defined by Simon. All tarsi clearly divided by a longitudinal band of bristles. On anterior tarsi this band is quite narrow, on the fourth tarsi almost as wide as the tarsus itself.

Neischnocolus panamanus gen. et sp. nov., 1 female from Cerro Iglesia.

Psalmopoeus pulcher sp. nov., 1 male collected by Dr. W. J. Baerg in the Canal Zone.

Psalmopoeus rufus sp. nov., 1 female—definite locality not given.

Sericopelma communis F. Cambridge, 1 male, 1 female, Canal Zone.

Sericopelma rubronitens Ausser, 2 females from Culebra, 2 females from Bocas del Toro.

Xenesthis immanis (Ausserer), occurs also in Colombia.

Family Barychelidæ

Merothele zebra gen. et sp. nov., 1 female from Cerro Flores.

Family Dipluridæ

Accola spinosa sp. nov., 1 female from Wilcox camp on San Lorenzo River.

**Evagrus guatemalensis* F. Cambridge, 1 immature specimen from the Wilcox camp on San Lorenzo River, found under wood, in forest. Previously known from Guatemala.

**Ischnothele guianensis* (Walckenaer), 1 male and 4 females from Santiago found in funnel-shaped webs along stream. Previously recorded from Guiana, Brazil, Costa Rica and Bahamas.

Family Ctenizidæ

Actinopus robustus (Cambridge).

Ancylotrypa panamana sp. nov., 1 female from the Wilcox camp on San Lorenzo River. First record of an American representative of this genus.

SUB-ORDER ARACHNOMORPHÆ

Family Uloboridæ

**Uloborus americanus* Walckenaer, 1 female from the Wilcox camp on San Lorenzo River. Reported from Guatemala, Costa Rica, West Indies (including Bahamas) and U. S. A.

Uloborus geniculatus (Oliver), 1 female from Cerro Flores. A cosmopolitan species.

Uloborus sp., 1 immature male from Remedios.

Family Acanthoctenidæ

Acanthoctenus maculatus sp. nov., 1 immature specimen from Santiago, 1 very young specimen from the Wilcox camp on San Lorenzo River, and 1 immature specimen from Ancon Hill, C. Z.

Family Zoropsidæ

Zorocrates fuscus Simon, known also from Guatemala and Mexico.

Family Dictynidæ

**Dictyna nivea* (Cambridge), 1 female from Sona. Previously known from Guatemala and Mexico.

Family Filistatidæ

Filistata hibernalis Hentz.

Family Sicariidæ

Loxosceles rufipes (Lucas), 1 female from Panama City, 3 females and 2 immature specimens from the Wilcox camp on San Lorenzo River. Occurs in tropical and sub-tropical North and South America.

Scytodes championi F. Cambridge, 2 females with eggs from Cerro Flores. Occurs also in Mexico, Guatemala and Costa Rica.

**Scytodes fusca* Walckenaer, 1 female from Puerto Royal near Montijo. Occurs in tropical South, Central and North America and the West Indies.

Scytodes longipes Lucas, 1 female with spiderlings from a crevice in a rock at Santiago, 1 female from Remedios, 1 female from the Wilcox camp on San Lorenzo River, 2 immature specimens from Cerro Flores, and 1 immature specimen from Ancon Hill, C. Z. Widely distributed neotropical species.

Family Oonopidæ

Oonops reticulatus sp. nov., 1 female from Panama City.

Family Caponiidæ

Caponina sp.?, 1 specimen from the Wilcox camp on San Lorenzo River. Only cephalothorax and appendages preserved. Carapace quite flat, yellow. The two eyes in a black spot. Three claws on all tarsi. Legs of the same color as carapace.

Family Drassidæ

Callilepis sp.?, 1 immature specimen from Ancon Hill, C. Z.

Family Hersiliidæ

Tama mexicana (Cambridge), known also from Costa Rica and Mexico.

Family Pholcidæ

**Artema atlanta* Walckenaer, 1 female from Colon. Reported from tropical South and North America and the West Indies.

**Modisimus inoratus* Cambridge, 3 females from the Wilcox camp on San Lorenzo River. Known from Costa Rica, Mexico and Jamaica.

**Physocyclus globosus* (Taczanowski), several specimens from Santiago, Remedios and Panama City. Known from tropical South and North America, West Indies and Southern U. S. A.

Smeringopus geniculatus (Thorell) = elongatus Vinson, cosmopolitan.

**Spermophora placens* Cambridge, 3 immature specimens from Ancon Hill, C. Z. Described from Mexico.

Family Theridiidæ

**Argyrodes argentatus* Cambridge, males and females from the webs of Nephila clavipes. Panama City, Ancon Hill, Remedios. Known from Brazil, Mexico and the East Indies, Ceylon and Madagascar.

Argyrodes cingulatus sp. nov., 1 female from the web of *Argiope argentata* in Panama City.

**Argyrodes jucundus* Cambridge, 1 female from the web of Nephila clavipes on Taboga Island. Recorded from Brazil, Guatemala, Galapagos Is. and California.

Ariamnes approximatus Cambridge.

Dipoena pulchella n. sp., 1 female from the Wilcox camp on San Lorenzo River.

Episinus putus Cambridge, occurs also in Mexico.

Euryopis lineatipes Cambridge, reported also from Guatemala and Mexico.

Latrodectus geometricus C. Koch, cosmopolitan.

Latrodectus mactans (Fabricius), 2 females from Las Palmas, widely distributed American species.

Lithyphantes lugubris F. Cambridge, recorded also from Mexico and Costa Rica.

Rhomphaea procera (Cambridge).

Spintharus flavidus Hentz, recorded also from U. S. A., Mexico, West Indies, Guatemala and Peru.

Theridion albulum Cambridge.

Theridion cambridgei Petrunkevitch (= vittatum Cambridge), recorded also from Guatemala and Mexico.

Theridion defunctum sp. nov., 1 male from the Wilcox camp on San Lorenzo River.

Theridion fordum Keyserling, recorded also from Brazil, Venezuela, Costa Rica, Guatemala, Mexico, and Texas.

**Theridion jucundum Cambridge,* 1 male and 1 female from Cerro Flores. Known from Mexico and Guatemala.

Theridion longistylum (F. Cambridge).

Theridion niveum Cambridge, known also from Mexico.

Theridion rubicundum (F. Cambridge).

Theridion rufipes Lucas, 2 females from the Wilcox camp on San Lorenzo River. Cosmopolitan.

Theridion studiosum Hentz, tropical and sub-tropical North and South America.

Theridion taeniatum Keyserling, known also from Venezuela, Costa Rica and Mexico.

Theridion tepidariorum C. Koch, cosmopolitan.

Theridion vituperabile Petrunkevitch (= blandum Cambridge), cosmopolitan.

Theridula opulenta (Walckenaer), cosmopolitan.

Thwaitesia affinis Cambridge, 1 female, 1 immature female and 1 immature male from the Wilcox camp on San Lorenzo River. Occurs also in Brazil.

Family Linyphiidæ

Erigone bimaculata Keyserling.

Gongylidiellum panamanum sp. nov., 1 female from the Wilcox camp on San Lorenzo River.

**Linyphia calcarifera* (Keyserling), 1 female from the Wilcox camp on San Lorenzo River, 1 female from Ancon Hill, C. Z. First described from Colombia.

Linyphia uncata (F. Cambridge), reported also from Guatemala and Mexico.

**Linyphia* sp.?, 1 immature male from Cerro Flores. PME's largest and far apart, AME's smallest and close together. Laterals subequal, separated from each other by a black line.

Sthelota albonotata (Keyserling).

Family Argiopidæ

Acacesia foliata (Hentz) known also from Guatemala, Costa Rica, West Indies, Mexico and U. S. A.

Acacesia cornigera sp. nov., 1 female, 1 male and 1 immature male from the Wilcox camp on San Lorenzo River, 1 immature specimen from Cerro Iglesia.

Araneus (*Verrucosa*) *arenatus* (Walckenaer), known also from Costa Rica, Guatemala, Haiti, Mexico and U. S. A.

Araneus (*Parawixia*) *armatus* (Cambridge), known also from Guatemala.

Araneus bispinosus (Keyserling), known also from California, Arizona and Haiti.

Araneus championi (Cambridge), known also from Guatemala.

Araneus concoloratus F. Cambridge.

Araneus consequus (Cambridge), know also from Mexico.

**Araneus* (*Wixia*) *clivosus* (Cambridge), 1 male from Trijoles, C. Z., first described from Mexico.

Araneus (*Parawixia*) *destrictus* (Cambridge), known also from Guatemala.

Araneus (*Eriophora*) *edax* (Blackwall), 1 female from the Canal Zone. Occurs also in Brazil, Colombia, Costa Rica, Nicaragua, Guatemala and Mexico.

Araneus (*Epeiroides*) *fasciolatus* (Cambridge), occurs also in Guatemala.

Araneus glabratus F. Cambridge.

**Araneus* (*Wixia*) *globosus* (F. Cambridge), several young specimens from the Wilcox camp on San Lorenzo River. Originally described from Mexico.

Araneus graphicus (Cambridge), known also from Guatemala.

Araneus gravabilis (Cambridge), known also from Costa Rica and Guatemala.

Araneus (*Metazygia*) *gregalis* (Cambridge), occurs also in Guatemala, Costa Rica and Brazil.

**Araneus* (*Parawixia*) *guatemalensis* (Cambridge), 1 immature female from the Wilcox camp on San Lorenzo River. Occurs also in Guatemala and Mexico.

Araneus (*Parawixia*) *hoxaeus* (Cambridge), occurs also in Guatemala.

Araneus (*Parawixia*) *hypocritus* (Cambridge), occurs also in Guatemala.

Araneus (*Metepeira*) *labyrintheus* (Hentz), widely distributed from Labrador to Patagonia including all islands.

Araneus laticeps (Cambridge), occurs also in Guatemala.

Araneus (*Verrucosa*) *mexicanus* (Lucas), occurs also in Costa Rica, Guatemala, Mexico, West Indies and Colombia.

Araneus (*Neoscona*) *nauticus* (L. Koch), cosmopolitan.

Araneus nigrocinctus F. Cambridge.

Araneus (*Neoscona*) *oaxacensis* (Keyserling), occurs also in Mexico, California, Cuba and St. Vincent, W. I.

Araneus pallidulus (Keyserling), occurs also in Colombia, Costa Rica and Curaçao, W. I.

Araneus (*Eriophora*) *purpurascens* (Cambridge), 1 female from the Canal Zone, occurs also in Venezuela, Colombia, Costa Rica, Guatemala, Mexico and St. Vincent, W. I.

Araneus (*Parawixia*) *rigidus* (Cambridge).

Araneus (*Wixia*) *rufus* (Cambridge), occurs also in Guatemala and Mexico.

Araneus (*Neosconella*) *solersioides* (Cambridge), 2 females from Panama City, occurs also in Costa Rica and Mexico.

Araneus spiniger (Cambridge), occurs also in Guatemala and Florida.

Araneus (*Wagneriana*) *tauricornis* (Cambridge), occurs also in Colombia, Guatemala, Mexico, Cuba, Haiti and Southern U. S. A.

Araneus (*Wagneriana*) *undecimtuberculatus* (Keyserling), occurs also in Colombia, Guatemala, Mexico, Cuba, Haiti, and Florida.

Araneus veniliae (*Keyserling*), occurs also in Brazil and Colombia.

Araneus (*Eriophora*) sp.?, 1 immature female from Remedios, several young specimens from Colon.

Araneus (*Wixia*) sp.?, 1 immature specimen from the Wilcox camp on San Lorenzo River.

Araneus sp.?, young specimens from Sona, Trijoles, C. Z., and San Lorenzo River.

Argiope argentata (Fabricius) numerous specimens including 1 male from Panama City and 1 male from Trijoles, C. Z.—widely distributed from Southern United States to Patagonia including all islands.

Argiope trifasciata (Forskäl), cosmopolitan.

Azilia guatemalensis Cambridge, occurs also in Guatemala, St. Vincent, W. I., Costa Rica, Venezuela and Peru.

Bertrana hieroglyphica sp. nov., 1 male from the Wilcox camp on San Lorenzo River.

Carepalxis gibbosa Cambridge, occurs also in Guatemala.

Cyclosa lacerta (Cambridge), occurs also in Guatemala.

Cyclosa V-notata sp. nov., 1 female from the Wilcox camp on San Lorenzo River.

Cyrtognatha globosa sp. nov., 1 female and 1 immature male from the Wilcox camp on San Lorenzo River.

Cyrtognatha lepida (Cambridge).

Cyrtognatha pachygnathoides (Cambridge).

Drexelia directa (Hentz), 1 immature female from Sona. Known also from Costa Rica, Guatemala, Mexico, Cuba and Southern U. S. A.

Edricus crassicauda (Keyserling), occurs also in Colombia, Guatemala, Mexico and Porto Rico, W. I.

Edricus spinigerus Cambridge, occurs also in Guatemala.

Eustala fragilis (Cambridge), occurs also in Guatemala.

Eustala fusco-vittata (Keyserling), occurs also in Colombia, Venezuela, Brazil, Paraguay, Costa Rica, Guatemala, Mexico and the West Indies.

Eustala guttata F. Cambridge, occurs also in Guatemala and Mexico.

Eustala latebricola (Cambridge), occurs also in Guatemala.

Eustala scutigera (Cambridge), occurs also in Costa Rica, Guatemala and Mexico.

Gasteracantha cancriformis (Linnaeus), 1 female from Fort Sherman, C. Z. Reported from Guiana, Brazil, Paraguay, Costa Rica, Guatemala, Mexico, West Indies and Southern U. S. A.

Gea panamensis Chamberlin.

Glyptogona decemtuberculata (Cambridge), occurs also in Guatemala.

Kaira altiventer Cambridge, occurs also in Guatemala.

Kaira gibberosa Cambridge, occurs also in Guatemala.

Leucauge acuminata (Cambridge), occurs also in Guatemala and Costa Rica.

Leucauge argyra (Walckenaer), widely distributed through subtropical and tropical North, Central and South America and the West Indies.

Leucauge curta (Cambridge).

Leucauge idonea (Cambridge), occurs also in Guatemala.

Leucauge moerens (Cambridge), occurs also in Costa Rica, Guatemala and Mexico.

Leucauge venusta (Walckenaer), occurs in U. S. A., Mexico, Central America, West Indies (including Bahamas), Bermuda, Colombia and Ecuador.

Leucauge sp.?, many immature specimens from Colon, Panama City, Sona, Remedios, San Lorenzo River, Ancon Hill, C. Z.

Mangora bimaculata (Cambridge), occurs also in Guatemala.

**Mangora trilineata* Cambridge, 1 female from Barro Colorado, 2 females from the Wilcox camp on San Lorenzo River. Described from Costa Rica, Guatemala and Mexico.

Mangora sp.?, 2 immature females and 1 immature male from the Wilcox camp on San Lorenzo River. A small species with black venter, black lip and maxillae. Abdomen above with two black shoulderspots with white between, two longitudinal white bands full length of abdomen and 2-2-1 white spots in middle. All eyes on black spots.

**Mecynometa globosa* (Cambridge), 1 female from the Wilcox

camp on San Lorenzo River. First described from Guatemala.

**Meta gravida* (Cambridge), 1 male, 8 females and 1 immature specimen from Cerro Flores. Occurs in Guatemala.

Metargyra debilis (Cambridge), 1 male from the Wilcox camp on San Lorenzo River. Occurs also in Costa Rica and Mexico.

Micrathena brevis (Cambridge), occurs also in Guatemala and Mexico.

Micrathena cornigera (Cambridge), occurs also in Guatemala.

**Micrathena crassa* (Keyserling), 2 females from Cerro Flores. Described from Colombia.

Micrathena duodecimspinosa (Cambridge), occurs also in Costa Rica, Guatemala and Mexico.

Micrathena fericula (Cambridge), occurs also in Guatemala.

Micrathena flaveola (Perty) (= *gladiola* Walck.), occurs also in Guatemala and Brazil.

Micrathena horrida (Taczanowski), occurs also in Guiana, Brazil, Peru, Paraguay, Venezuela, Guatemala, Mexico and Jamaica.

Micrathena longicauda (Cambridge), occurs also in Guatemala and Mexico.

Micrathena mammillata (Butler), occurs also in Brazil, Costa Rica, Guatemala and Mexico.

Micrathena sexspinosa (Hahn) (= *obtusospina* Keys), a widely distributed species throughout Central America, West Indies, Mexico, and tropical South America.

Micrathena parallela (Cambridge), occurs also in Guatemala.

Micrathena patruelis (C. Koch), widely distributed throughout South and Central America, reaching into Mexico.

**Micrathena quadriserrata* F. Cambridge, 1 female from Trijoles, C. Z. Recorded from Guatemala and Venezuela.

Micrathena serrata F. Cambridge.

**Micrathena striata* F. Cambridge (described from Guatemala), 1 female from Trijoles, C. Z.

Micrathena vitiosa (Cambridge), occurs also in Guatemala.

Mimicosa spinosa gen. et sp. nov., 2 males and 2 immature males from Trijoles, C. Z.

Nephila clavipes (Linnaeus), females from the Canal Zone, Remedios, and Taboga Island. Occurs throughout tropical and sub-tropical North, Central and South America, including all islands.

Pseudometa alboguttata (Cambridge), occurs also in Costa Rica, Guatemala and Mexico.

Scoloderus americanus (Cambridge).

Scoloderus gibber Cambridge, occurs also in Guatemala and Mexico.

Scoloderus tuberculifer (Cambridge), occurs also in Guatemala and Florida.

Tetragnatha alba F. Cambridge, occurs also in Costa Rica, Guatemala and Mexico.

**Tetragnatha antillana* Simon, 1 male and 2 females from the Wilcox camp on San Lorenzo River. Occurs in Costa Rica, Guatemala, Mexico, Porto Rico and St. Vincent, W. I.

Tetragnatha tenuis Cambridge, occurs also in Guatemala.

Tetragnatha tenuissima Cambridge, 1 male and 3 females from the Wilcox camp on San Lorenzo River. Occurs also in Costa Rica, Guatemala and Mexico.

Tetragnatha sp.?, immature specimens belonging to various species from the Wilcox camp on San Lorenzo River and Cerro Iglesia.

Theridiosoma globosum (Cambridge), recorded from Central America and Mexico.

Family Mimetidæ

Gleanor consequus Cambridge.

Gelanor distinctus Cambridge.

Gelanor heraldicus sp. nov., 1 female and 1 immature specimen from the Wilcox camp on San Lorenzo River.

Gelanor mixtus Cambridge, 1 immature specimen from the Wilcox camp on San Lorenzo River. Occurs also in Guatemala.

Mimetus rapax Cambridge, occurs also in Costa Rica.

Mimetus trituberculatus Cambridge.

Family Thomisidæ

Isaloides putus (Cambridge), occurs also in Mexico.

Misumenoides blandus (Cambridge), occurs also in Guatemala.

Misumenoides magnus (Keyserling), occurs also in Colombia, Costa Rica, Guatemala and Mexico.

Misumenoides rugosus (Cambridge), occurs also in Guatemala.

Misumenoides tibialis (Cambridge).

**Misumenops celer* (Hentz) (= *spinosa* Keys), 1 male from Trijoles, C. Z. occurs in the Southern U. S. A. to California and Washington, Mexico, Cuba and Porto Rico.

Misumenops damnosus (Keyserling), occurs also in Guatemala and Mexico.

Misumenops pallens (Keyserling), occurs in Guatemala, Colombia, Brazil, Peru and Chile.

Misumenops pascalis (Cambridge).

Onocolus pentagonus (Keyserling), occurs in Brazil.

Parastephanops cognatus (Cambridge).

**Philodromus* sp.?, 1 immature specimen from Cerro Flores.

Strophius hirsutus Cambridge, occurs also in Costa Rica.

**Strophius* sp.?, 2 immature specimens from the Wilcox camp on San Lorenzo River.

Synæma adjunctum Cambridge.

Synæma affinitatum Cambridge, occurs also in Costa Rica, Guatemala and Mexico.

Synæma fuscomandibulatum sp. nov., 2 males from Cerro Flores.

Synæma maculosum Cambridge, 1 male from Cerro Flores, known also from Costa Rica and Guatemala.

Synæma palliatum Cambridge.

Synæma socium Cambridge.

Tmarus corruptus Cambridge, occurs also in Mexico.

Tmarus decens Cambridge.

Tmarus ineptus Cambridge.

Tmarus intentus Cambridge, occurs also in Guatemala.

Tmarus mundulus Cambridge.

Tmarus pauper Cambridge.

Tmarus studiosus Cambridge.

**Tmarus* sp.?, 1 immature female from the Wilcox camp on San Lorenzo River.

Tobias rugosus (Taczanowski), occurs also in Guiana, Brazil, Peru and Haiti.

**Xysticus* sp.?, 1 immature female from Cerro Flores.

Family Selenopidæ

**Selenops aissus* Walckenaer (nec Keyserling), 1 female from Remedios, 1 female with egg cocoon from Sona (March 28). Occurs also in the Bahamas and in Cuba, Jamaica, Martinique.

Selenops mexicanus Keyserling, occurs also in Costa Rica. Nicaragua, Guatemala and Mexico.

**Selenops* sp.?, 1 immature specimen, possibly *S. mexicanus* from Panama City.

Family Sparassidæ

Defectrix defectrix gen. et sp. nov., 1 immature female from Panama City.

Heteropoda venatoria (Linnaeus), cosmopolitan.

Olios obscurus (Keyserling), occurs also in Costa Rica and Mexico.

Prusias nugalis Cambridge, occurs also in Mexico.

Prusias semotus (Cambridge).

Family Clubionidæ

Anyphæna plana F. Cambridge, occurs also in Costa Rica.

Anyphæna stylifer F. Cambridge.

Arachosia puta Cambridge.

Aysha simplex Cambridge.

Aysha valvula F. Cambridge.

Chiracanthium ferum Cambridge, occurs also in Mexico.

Clubiona sigillata sp. nov., 1 male, 1 female from the Wilcox camp on San Lorenzo River.

**Corinna bulbosa* F. Cambridge, 1 female from the Wilcox camp on San Lorenzo River, known from Guatemala and Mexico.

Corinna bulbula F. Cambridge.

Corinna luteomaculata sp. nov., 1 female from Cerro Flores, on the ground in the forest.

Corinna mucronata F. Cambridge, occurs also in Costa Rica.

Corinna nervosa F. Cambridge, occurs also in Guatemala.

**Corinna* sp.?, 2 immature specimens from Cerro Flores.

Cragus mandibulatus sp. nov., 1 male, 1 female from the Wilcox camp on San Lorenzo River, in clearing.

**Cragus pallidus* Cambridge, 1 male from the Wilcox camp on San Lorenzo River, on shrubs in forest, known from Guatemala.

Eutichurus putus Cambridge.

**Megalostrata mordicans* (Cambridge), 1 male from Cerro Flores, first described from Guatemala.

**Myrmecotypus dubius* (Cambridge), 1 immature female, from Bocas del Monte, 1 quite young specimen from the Wilcox camp on San Lorenzo River, first described from Mexico.

**Myrmecotypus* sp.?, 1 immature female and 4 quite young specimens from the Wilcox camp on San Lorenzo River.

Sillus curvispina F. Cambridge.

**Sillus longispina* F. Cambridge, 1 male and 1 immature female from the Wilcox camp on San Lorenzo River, 1 young

specimen from Remedios. Occurs also in Costa Rica and Guatemala.

Sillus placens (Cambridge).

Sphecotypus niger (Perty), occurs also in Brazil and Venezuela.

Syrisca hirsuta sp. nov., 1 male from Cerro Iglesia, 1 female from Panama City.

Syrisca pulchra sp. nov., 1 female from Remedios.

Teudis roseus F. Cambridge.

Trachelas bispinosus F. Cambridge, 1 female from Cerro Flores, occurs also in Costa Rica.

**Trachelas* sp.? (possibly *bispinosus*), 2 immature females from Cerro Flores.

Family Ctenidæ

Celætycheus paradoxus F. Cambridge.

Ctenus curvipes (Keyserling).

Ctenus rubripes Keyserling, occurs also in Ecuador.

**Ctenus sinuatipes* F. Cambridge, 1 male from Cerro Flores, 1 male from Culebra. The latter is considerably larger, but exhibits the same structure. First described from Costa Rica.

Ctenus W-notatus sp. nov., 1 female from the Wilcox camp on San Lorenzo River.

**Ctenus* sp.?, several immature specimens of different age from Cerro Flores, Santiago and the Wilcox camp on San Lorenzo River. This is a small species with yellow median band on carapace.

**Ctenus* sp.?, several immature specimens from the Wilcox camp on San Lorenzo River. A larger species with a black median broad band on carapace and abdomen and with a black longitudinal band on cheliceræ.

**Ctenus* sp.?, 1 immature specimen from Cerro Iglesia.

Cupiennius coccineus F. Cambridge, recorded also from Costa Rica.

Cupiennius foliatus F. Cambridge, occurs also in Costa Rica and Nicaragua.

Cupiennius sallei (Keyserling), occurs also in Costa Rica, Honduras, Mexico and Florida.

Family Agelenidæ

Agelena panamana sp. nov., 1 female from Bocas del Monte, 1 female from the Wilcox camp on San Lorenzo River.

Tegenaria domestica (Clerck), cosmopolitan.

Family Pisauridæ

Ancylometes bogotensis (Keyserling), occurs also in Colombia, Brazil, Bolivia and Costa Rica.

Ancylometes valentinei sp. nov., 1 male and 1 female and 1 immature female from the Wilcox camp on San Lorenzo River.

**Ancylometes* sp.?, 1 immature male and 1 immature female from the Wilcox camp on San Lorenzo River. This is a larger species than *A. valentinei.* Total length of the immature male 25 mm. Legs shorter and stouter, scopulæ green iridescent. On 3d and 4th tarsi five or six pairs of short spines below.

Dyrines lineatipes sp. nov., 1 male from the Wilcox camp on San Lorenzo River.

Enna approximata (Cambridge).

Enna jullieni (Simon), occurs also in Venezuela.

Enna minor sp. nov., 1 female from Santiago.

**Hesydrus habilis* (Cambridge), 1 male from Remedios, known from Costa Rica and Guatemala.

Syntrechalea tenuis F. Cambridge.

Thaumasia uncata F. Cambridge.

Trechalea magnifica sp. nov., 2 females and several immature specimens of various ages from the Wilcox camp on San Lorenzo River, Bocas del Monte, La Mesa and Santiago.

Family Lycosidæ

Arctosa cheluncata sp. nov., 2 males, 3 females and 1 immature female from Santiago, 1 female from Remedios, 1 immature female from La Mesa, and 1 male, 1 female and 1 immature female from the Wilcox camp on San Lorenzo River.

Arctosa panamana sp. nov., 1 female from Remedios.

**Arctosa trifida* F. Cambridge, 1 immature male and 1 immature female from Remedios, known from Costa Rica, Guatemala and Mexico.

Lycosa cerrofloresiana sp. nov., 1 male from Cerro Flores.

**Lycosa dilatata* F. Cambridge, 2 large females of the yellowish red variety from Culebra, 1 female of the typical variety from Bocas del Monte, 1 ditto from Cerro Flores, 1 female with black venter from Cerro Flores, 2 ditto from Bocas del Monte. First described from Mexico.

Lycosa fusca (Keyserling), known from Central America, Cuba and Porto Rico.

Lycosa longitarsis F. Cambridge, 1 female from Culebra, occurs in Costa Rica, Guatemala and Mexico.

Lycosa pugnatrix (Keyserling), recorded from "Central America" without definite locality.

Lycosa rabida Walckenaer, 1 male from Culebra, several males and females from Bocas del Monte. Common in U. S. A. and Mexico.

Lycosa sanlorenziana sp. nov., 1 female and 1 immature specimen from the Wilcox camp on San Lorenzo River.

Pardosa albopilosa sp. nov., 1 male from Panama City, 1 male from Remedios.

**Pardosa uncatula* F. Cambridge, 1 male and 1 immature specimen from the Wilcox camp on San Lorenzo River. Known from Costa Rica, Guatemala and Mexico.

**Pardosa* sp.?, 2 immature specimens from Sona.

Family Senoculidæ

Senoculus canaliculatus F. Cambridge, occurs also in Guatemala and Mexico.

Senoculus purpureus (Simon).

Senoculus valentinei sp. nov., 2 males from the Wilcox camp on San Lorenzo River.

Family Oxyopidæ

Oxyopeidon flebile Cambridge.

Oxyopeidon putum Cambridge, 1 female from the Wilcox camp on San Lorenzo River, occurs also in Costa Rica.

Oxyopes clypeatus F. Cambridge.

Oxyopes globosus F. Cambridge.

Oxyopes salticus Hentz, a widely distributed species found in the United States as far north as New York, in Mexico, Central America, West Indies, Brazil, Colombia and Bolivia.

Oxyopes tricuspidatus F. Cambridge.

**Oxyopes* sp.?, 1 immature specimen from Sona.

Peucetia thalassina (C. Koch), recorded from Central America without locality. and from Mexico.

Peucetia rubricapilla sp. nov., 1 female and several young specimens from Sona.

Family Attidæ

Amycus benignus (Peckham), occurs also in Guatemala and Mexico.

Amycus fallax Peckham.

Amycus hieroglyphicus Peckham, occurs also in Mexico.

Amycus pacatus Peckham, from Central America without definite locality.

Amycus palpinalis F. Cambridge, 1 male, 1 young specimen from the Wilcox camp on San Lorenzo River.

Amycus quadriguttatus F. Cambridge, occurs also in Guatemala and Mexico.

**Amycus spiralifer* F. Cambridge, 1 male from the Wilcox camp on San Lorenzo River, occurs also in Guatemala.

Avitus diolenii Peckham.

Balmaceda punctata Peckham, from "Central America" without definite locality.

Beata cephalica F. Cambridge.

Beata flavolineata (F. Cambridge).

Beata longipes (F. Cambridge).

Beata magna Peckham, 1 female from the Wilcox camp on San Lorenzo River. Occurs also in Guatemala and Colombia.

Beata striata sp. nov., 1 female from Cerro Iglesia.

**Beata variegata* (F. Cambridge), 1 male from Trijoles, Canal Zone, April 28, 1919, occurs also in Mexico.

**Beata sp.?*, 1 immature female from the Wilcox camp on San Lorenzo River.

**Breda milvina* (C. Koch), 1 female from Cerro Iglesia, occurs in Brazil and Trinidad.

Capidava nigropicta (F. Cambridge), occurs also in Costa Rica.

**Chapoda inermis* (F. Cambridge), 1 female from Cerro Flores, occurs also in Costa Rica and Mexico.

**Chapoda* sp.?, 1 young specimen from the Wilcox camp on San Lorenzo River.

Chira designata Peckham.

Cobanus extensus (Peckham).

Cobanus flavens (Peckham).

Cobanus mandibularis (Peckham), occurs also in Costa Rica.

**Cobanus subfuscus* F. Cambridge, 1 male from Cerro Flores, occurs also in Costa Rica.

Cobanus unicolor F. Cambridge, occurs also in Costa Rica.

Colonus crucifer F. Cambridge.

Corythalia albicincta (F. Cambridge) occurs also in Costa Rica and Guatemala.

Corythalia bicincta sp. nov., 2 males and 1 female from the Wilcox camp on San Lorenzo River.

Corythalia nigriventer (F. Cambridge).

Corythalia pulchra sp. nov., 1 female from Panama City, 1 female from Fort Sherman, C. Z.

Corythalia panamana sp. nov., 1 male from Remedios, 1 male and several young from Panama City.

Corythalia quadriguttata (F. Cambridge), occurs also in Guatemala and Mexico.

Corythalia spiralis F. Cambridge, 1 male from the Wilcox camp on San Lorenzo River, occurs Costa Rica, Guatemala and Mexico.

Corythalia spirorbis (F. Cambridge).

Corythalia sulphurea (F. Cambridge), 6 females and 3 males from Cerro Flores, 2 males from the Wilcox camp on San Lorenzo River.

Corythalia voluta (F. Cambridge).

**Corythalia* sp.? (possibly *sulphurea*), 2 immature specimens from Bocas del Monte and 3 from the Wilcox camp on San Lorenzo River.

Cylistella castanea sp. nov., 1 female from the Wilcox camp on San Lorenzo River.

Cylistella scarabaeoides (Cambridge), occurs also in Mexico.

**Cylistella* sp.?, 1 immature specimen from the Wilcox camp on San Lorenzo River.

Dendryphantes (*Phidippus*) *aureopilosus* (F. Cambridge).

Dendryphantes (*Metaphidippus*) *bicavatus* (F. Cambridge).

Dendryphantes (*Metaphidippus*) *bispinosus* (F. Cambridge), occurs also in Costa Rica and Guatemala.

Dendryphantes (*Phiddippus*) *carneus* (Peckham), Central America without definite locality.

Dendryphantes (*Metaphidippus*) *centralis* (Peckham).

Dendryphantes (*Metaphidippus*) *cupreus* (F. Cambridge).

Dendryphantes (*Metaphidippus*) *expallidatus* (F. Cambridge).

Dendryphantes (*Metaphidippus*) *iridescens* (F. Cambridge).

Dendryphantes (*Metaphidippus*) *laetabilis* Peckham.

**Dendryphantes* (*Metaphidippus*) *lanceolatus* (F. Cambridge), 1 male from Cerro Flores, occurs in Costa Rica, Guatemala and Mexico.

Dendryphantes (*Metaphidippus*) *levispinus* (F. Cambridge).

Dendryphantes (*Metaphidippus*) *longipalpus* (F. Cambridge).

Dendryphantes (*Metaphidippus*) *momus* (F. Cambridge), occurs also in Guatemala.

**Dendryphantes* (*Metaphidippus*) *octonotatus* (F. Cambridge),

3 immature specimens from Cerro Flores, occurs also in Costa Rica and Guatemala.

Dendryphantes (*Metaphidippus*) *quadrinotatus* (F. Cambridge), occurs also in Costa Rica.

Erica eugenia Peckham, occurs also in Brazil and Peru.

Evophrys newtoni Peckham, recorded from "Central America" without definite locality.

Freya bicavata (F. Cambridge).

Freya bifida (F. Cambridge).

Freya bifurcata (F. Cambridge), 1 male from Panama City.

Freya bilobata (F. Cambridge), occurs also in Guatemala.

Freya bulbosa (F. Cambridge).

Freya distincta sp. nov., 1 male from the Wilcox camp on San Lorenzo River.

Freya grisea (F. Cambridge), occurs also in Guatemala.

Freya infuscata (F. Cambridge).

**Freya longispina* (F. Cambridge), 1 male from the Wilcox camp on San Lorenzo River, occurs in Guatemala.

Freya minuta (F. Cambridge).

**Freya* sp.?, 1 immature specimen from Cerro Flores and one from Cerro Iglesia.

Hasarius adansoni (Audouin), cosmopolitan.

Lyssomanes elegans F. Cambridge, occurs also in Mexico.

Lyssomanes jemineus Peckham, Central America and South America, definite localities not given.

Lyssomanes patens Peckham, occurs also in Honduras.

Lyssomanes remotus Peckham.

Lyssomanes robustus (Taczanowski), described from Peru, recorded as occurring in Central America, but without definite locality.

Lyssomanes simpliceps F. Cambridge, occurs also in Guatemala.

Lyssomanes trifurcatus F. Cambridge.

Lyssomanes viridis (Walckenaer), a widely distributed species throughout Southern U. S. A., Central America and the West Indies.

Menemerus bivittatus (Dufour), cosmopolitan.

Mica longithorax gen. et sp. nov., 1 female from Trijoles, Canal Zone.

Myrmarachne centralis (Peckham), occurs also in Mexico.

Myrmarachne championi (F. Cambridge).

Paradamoetas formicina Peckham, occurs also in Guatemala and Texas.

Partona roseus (F. Cambridge).

Peckhamia variegata (F. Cambridge).

Pensacola radians (Peckham).

Phiale bicuspidata (F. Cambridge), 1 female from the Wilcox camp on San Lorenzo River, occurs also in Costa Rica.

Phiale delecta (Peckham), 1 female from Remedios, occurs also in Costa Rica, Guatemala and Mexico.

Phiale elegans (F. Cambridge).

Phiale flavescens (Peckham).

Phiale geminata (F. Cambridge).

Phiale gratiosa C. Koch, occurs also in Brazil and Peru.

Phiale interrupta (F. Cambridge), occurs also in Costa Rica and Guatemala.

Phiale mimica (C. Koch), occurs also in Brazil.

Phiale niveo-guttata (F. Cambridge).

Phiale panamense Chamberlin.

**Phiale simplicicava* (F. Cambridge), 2 females from Panama City, 1 immature male from the Wilcox camp on San Lorenzo River, occurs in Mexico.

**Phiale* sp.?, (possibly *simplicicava*), several young specimens from Cerro Iglesia, Cerro Flores and the Wilcox camp on San Lorenzo River.

Plexippus paykulli (Audouin), cosmopolitan.

Salticus albocinctus (Peckham), "Central America" without definite locality, Mexico, also Texas and Utah of U. S. A.

Sidusa recondita (Peckham), occurs also in Guatemala.

Simonella bicolor Peckham, occurs also in Venezuela.

Thiodina sylvana (Hentz), occurs also in Costa Rica, Guatemala, Mexico, Southern U. S. A., and Cuba.

*? *Tutelina* sp.?, 1 young specimen from the Wilcox camp on San Lorenzo River.

Zygoballus desidiosus (Peckham), occurs also in Costa Rica.

Zygoballus maculatipes sp. nov., 1 female from Sona, 1 female from the Wilcox camp on San Lorenzo River.

Although the above list clearly indicates our comparative ignorance of the spider fauna of Panama, it is sufficiently extensive to permit a preliminary tabulation and to draw some interesting conclusions.

Family	Number of species recorded only from Panama	Number of species found only in Panama and Central Am.	Number of species found only in Panama and South Am.	Number of species cosmopolitan or neo-tropical	Total number of species found in Panama
Theraphosidæ	8	2	1	1	12
Barychelidæ	1	0	0	0	1
Dipluridæ	0	1	0	1	2
Ctenizidæ	2	0	0	0	2
Uloboridæ	0	1	0	1	2
Acanthoctenidæ	1	0	0	0	1
Zoropsidæ	0	1	0	0	1
Dictynidæ	0	1	0	0	1
Filistatidæ	0	0	0	1	1
Sicariidæ	0	1	0	3	4
Oonopidæ	1	0	0	0	1
Caponiidæ	1	0	0	0	1
Drassidæ	0	0	0	0	0
Hersiliidæ	0	1	0	0	1
Pholcidæ	0	2	0	3	5
Theridiidæ	8	7	1	11	27
Linyphiidæ	3	1	1	0	5
Argiopidæ	15	55	1	25	96
Mimetidæ	4	2	0	0	6
Thomisidæ	12	10	1	3	26
Selenopidæ	0	2	0	0	2
Sparassidæ	2	2	0	1	5
Clubionidæ	14	10	1	0	25
Ctenidæ	3	4	1	0	8
Agelenidæ	1	0	0	1	2
Pisauridæ	7	1	1	1	10
Lycosidæ	5	7	0	0	12
Senoculidæ	2	1	0	0	3
Oxyopidæ	5	2	0	1	8
Attidæ	47	40	6	6	99
Total	142	154	14	59	369

We see that out of the 369 species of spiders recorded as occurring in Panama 59 species are either Pan-American, or neo-tropical and widely distributed throughout the tropical belt of North, Central and South America, or else are cosmopolitan. Even if we were to regard these species as characteristic of the South American fauna—an assumption which were not quite warranted without a very extensive study of the distribution of each of the above species—and if we added to them the 14 species

recorded only from Panama and South America, we would have to place against these 73 species the 154 species, recorded only from Panama and other Central American countries, Mexico and the Southern United States, and the 142 species recorded only from Panama. It would require a great deal of new data to invalidate the unmistakable conclusion that *the spider Fauna of Panama is Central American in its character.* Moreover there is not a single family which would represent an exception from the general rule. Even the Sub-Order of Mygalomorphæ is Central American in character, though on the whole very poorly represented. Of the Family Uloboridæ among the Cribellatæ one species is Central American, the other cosmopolitan. The single species belonging to the Family Filistatidæ has a very wide distribution from the Southern United States to Argentina. Two families, the Attidæ and Argiopidæ, together comprise more than half of the total number. Of the 99 species of Attidæ 87 have not been recorded from South America, nor have been 70 species of the 96 Argiopidæ. As stated before, the analysis of species common to Panama and the West Indies will have to be postponed until later.

Five new genera and 46 new species together with several species inadequately described by former investigators are described in detail in the following pages.

SUB-ORDER MYGALOMORPHÆ

Family Theraphosidæ

Neischnocolus gen. nov.

Carapace low. Thoracic groove very slightly procurved. Eye-tubercle low, much wider than long. AME slightly smaller than ALE. PME smaller than AME and slightly smaller than PLE. Anterior row of eyes slightly procurved, posterior row recurved. Lateral eyes separated by less than radius. Lip wider than long, with few granules. Legs 4123, with spines. Stridulating organs wanting. Scopulæ divided longitudinally by setæ only on 3d and 4th tarsi. First metatarsus barely longer than tarsus; fourth metatarsus much longer, as long as tibia. Scopula on fourth metatarsus wanting. A single apical spine on first metatarsi, three apical spines on second, third and fourth

metatarsi. Closely related to *Ischnocolus* from which it may be distinguished by the intact scopula on anterior four tarsi, by the comparative shortness of first metatarsi and the complete absence of a scopula on fourth metatarsi. Type **N. panamanus.**

Neischnocolus panamanus sp. nov. Female. Total length with chelicerae 25.0 mm. Carapace flat, 8.8 mm. long, 7.3 mm. wide, anteriorly truncated squarely, with eyetubercle almost, but not quite reaching the margin. Thoracic groove very slightly procurved. Cephalothoracic sulci poorly developed, but visible. Eyetubercle low, eyearea in the shape of a transverse rectangle. AME slightly smaller than ALE, same size as PLE. PME slightly smaller than PLE, contiguous with them and subcontiguous with AME. Laterals distinctly, but slightly separated from each other. AME separated from each other by their radius. AME separated from ALE less than from each other. Anterior row of eyes slightly procurved by centers, posterior row recurved by centers. Carapace clothed with woolly grey hair. Stridulating organs wanting. Promargin of *chelicerae* with a row of 9 black teeth. (There is one additional black granule between fourth and fifth tooth on left chelicera, and two additional granules on right chelicera, one between the third and the fourth, the other between the fourth and fifth tooth.) Between the margins near base a small row of minute granules and others irregularly scattered. Both margins with a scopula, but the one of the promargin is the denser one. *Lip* wider than long in ratio 16:11, wider behind than in front, semicircular at base, emarginate at end, with a few granules. *Maxillae* with a few granules at base near inner angle. Immediately behind the lip a pair of smooth sclerites in every respect similar to sigilla. *Sternum* longer than wide in ratio 42:40. Three pairs of sigilla, first and second minute, third twice their size, subrotund, widely separated from each other and separated by their diameter from the margin of the sternum. Coxae subequal in size. A pad or brush on retrolateral surface of fourth leg wanting. Legs 4123. First leg 21 mm. long, second—18 mm., third—18 mm., fourth—24 mm. Third and fourth tarsal scopulae bisected by a row of setae, the setae on the fourth tarsus forming quite a wide band. First and second tarsal scopulae entire. First and second metatarsi barely longer than tarsi, third metatarsus distinctly longer,

fourth much longer than tarsus, as long as tibia. First metatarsus scopulate in distal 2/3, second in distal 1/2, third in distal 1/4, fourth without scopula. Three ventral apical spines and one prolateral on tibia of palp.

Spines on legs: I—tibia, ventral 2 near end; metatarsus—ventral 1 near end. II—tibia—ventral 1 near end, 1 before middle, prolateral 1 a little beyond middle. Metatarsus—ventral 3 at end. III—Tibia—ventral 3 at end, prolateral 1 beyond middle. Metatarsus—ventral 3-2-3, prolateral 2, retrolateral 2. IV—tibia —ventral 3 at end, prolateral 2, retrolateral 1. Metatarsus—ventral numerous spines, prolateral 2, retrolateral 2. Long trichobothria in two rows on all tibiæ, metatarsi and tarsi. Little club-shaped hairs on all tarsi between the common hairs. Legs clothed with short recumbent grey and long dark hair. Claws entirely concealed, curved, with a single row of five small teeth. Color in alcohol generally yellowish-brown, femora darker. Abdomen brown. One apparently mature female from Cerro Iglesia.

Sericopelma rubronitens Ausserer.

Large female from Culebra. Total length with cheliceræ 45 mm. Carapace 19 mm. long, 17 mm. wide, flat, with deep transverse groove and deep radiating sulci. Anteriorly carapace truncated almost squarely. Eyegroup transversely rectangular. Anterior row procurved by centers, posterior row recurved. AME separated from each other by slightly more than their radius and by almost their diameter from ALE. Laterals equal in size, larger than AME, separated from each other by 1/2 their long axis. PME smallest, about same distance from AME as from PLE, so that a straight line may be drawn touching these three eyes. Quadrangle much wider than long, trapeze-shaped. Tubercle not reaching edge of carapace, so that in front of the tubercle is a narrow true clypeus. Carapace clothed with light grey, woolly hair, with longer brown hairs interspersed. A fringe of long grey hair along anterior edge of carapace. A few long, stiff, brown hairs in front of and on eyetubercle. Stridulating organs wanting. Both margins of *chelicerœ* with a red brown scopula. Promargin with eleven powerful black teeth, with numerous black granules to the outside of the proximal three teeth. Retromargin smooth. *Lip* wider than long in

ratio 35:30, convex, narrowed toward end which is slightly emarginate, and covered with numerous brown granules and long, stiff, incurved hairs. Base of lip semicircular. Immediately behind lip two crescentic brown, shiny spots or sclerites on the sterum, resembling the sigilla, but much larger. *Maxillæ* with slightly but distinctly produced inner angle, with numerous granules at base. Scopula composed of long, fiery red hair. *Sternum* slightly longer than wide. Two pairs of sigilla, first pair opposite second coxæ, second opposite third coxæ, the two of both pairs wide apart, but separated from margin only by their length. Sternum clothed with woolly hair of white color and with stiff, erect, dark hair. Fourth coxæ considerably larger than third and distinctly larger than first and second. *Abdomen* clothed with white woolly hair and long hair which is dark at base, but becomes grey toward end. This hair is considerably longer on the dorsum than on the venter. In front of the genital groove is an oval of white woolly hair surrounded by long, dark brown hair. *Legs* 4123. First leg—53 mm., second—51 mm., third—51 mm., fourth—66 mm. Scopula on all tarsi heavy. On tarsi 1-3 it is entire, but on fourth tarsus it is bisected longitudinally by a narrow line of black bristles. First and second metatarsi with scopula almost to base, third and fourth without scopula. Several ventral spines on first and second tibia and one at base and three at end on second metatarsus. Several ventral spines on third and fourth tibia and on third metatarsus, while on fourth metatarsus the ventral spines are numerous. No spines on tarsi, nor anywhere else, except on palp, where there is one prolateral spine on femur near end and several ventral spines on tibia. Scopula of terminal joint entire. Femora of fourth pair with a velvety pad on retrolateral surface. Claws with three small teeth. Palpal claw smooth. Color in alcohol generally coffee-brown.

Psalmopoeus pulcher sp. nov. (Figure 2).

Male from the Canal Zone. Total length with cheliceræ 38 mm. Carapace 15 mm. long, 15 mm. wide, very flat. Head flat. Thoracic groove small, transverse, straight. Eyetubercle low, wider than long in ratio 39:30. Eyearea rectangular, laterals extending to edge of tubercle. Anterior row very slightly procurved, posterior row slightly recurved. AME largest,

separated from each other by almost their diameter and from ALE by less than their radius. PME smallest, contiguous with PLE, but widely separated from each other. Quadrangle much wider behind than in front and much wider than long. Anterior declivity of tubercle extending to edge of carapace which is therefore slightly bulging out in middle. True clypeus wanting, but distance between AME and the anterior edge of the eyetubercle

FIGURE 2, **Psalmopoeus pulcher** sp. nov., male. Left coxa of pedipalp with lyra.

is equal to the diameter of the AME. Several long thick, black hairs between the AME and in front of them. Elsewhere carapace is clothed with stiff dark and recumbent short, fine, grey hair, as are also the appendages and the dorsum of the abdomen. On the patellæ, tibiæ and metatarsi the black hair forms on the dorsal surface three indefinite longitudinal bands. Bushy lateral brushes on both sides of all patellæ, tibiæ, metatarsi and tarsi as well as of the third femora, while on first and second femora only a retrolateral brush is present, and on fourth femora only a prolateral one. Outer surface of *cheliceræ* with numerous parallel, transverse, shallow grooves and ridges and a few heavy, spinelike hairs below the scopula of the retromargin. Promargin with a row of 10 teeth of which the third and fifth from base are smaller than the rest. Both pro- and retromarginal scopulæ composed of fiery red hair. Lip black, with black hair, but chitin reddish beyond the granules. The *lip* is wider than long

in ratio 29:25. As the end of the lip, so is the inner angle of the maxillæ covered with numerous granules. The lyra is on the prolateral surface of the coxa of the pedipalp and is composed of from 14 to 16 bacilli (14 on the right coxa, 16 on the left), increasing in length distally. Beyond the last long bacillus are numerous short bacilli forming irregular rows as shown in Figure 2, overlapping each other and partly concealed by hair. The scopula of the maxillæ is fiery red. *Sternum* a little longer than wide, thickly clothed with black hair. Two pairs of sigilla, one pair opposite third coxæ, the other opposite fourth coxæ. This second pair is much larger than the first, long and submarginal. *Legs* 1423. First leg—62 mm., second—58 mm., third—51 mm., 4th—62 mm. First and second metatarsi and tarsi much the heaviest. First metatarsus 1 1/2 times as long as first tarsus. Fourth metatarsus more than twice as long as fourth tarsus, as long as fourth tibia. First tibia with two hooks covered with hair. No spines on legs. Tarsal scopulæ entire on all legs. First and second metatarsi scopulate to base, third metatarsus in distal half, fourth metatarsus in distal third. Claws so concealed by ungual tufts that they are quite invisible. Terminal joint of palp with a bushy pad. Bulb long, fine, curved and pointed, of the general appearance as in *P. cambridgei* with which *P. pulcher* has much in common except as to proportions.

Psalmopoeus rufus sp. nov.

Female. Total with cheliceræ 33 mm. Carapace flat, 13.5 mm. long, 12.5 mm. wide, anteriorly truncated square except for the slightly protruding eyetubercle which reaches the margin. Thoracic groove deep, transverse. Sulci well visible, but not deep. Eyearea in the shape of a transverse rectangle. Anterior row of eyes slightly procurved, posterior row slightly recurved. AME as large as ALE, separated from each other by a fraction more than their radius (in ratio 5:4.5) and from ALE by less than radius. PME contiguous with PLE, but widely separate from each other. Laterals slightly separated from each other. Quadrangle wider behind than in front, trapeze-shaped, much wider than long. PME smallest, PLE slightly smaller than ALE. Carapace clothed with white woolly hair with long white hair interspersed. Abdomen clothed above with short white,

short brown and very long white hair with darker base. Promargin of *cheliceræ* with 9 black teeth of which the second and fourth from base are smallest. Prolateral surface of cheliceræ smooth, red. Retrolateral surface red, clothed with short hair which becomes heavier and longer on the dorsal surface especially toward the base of the fang. Lyra on the coxa of the pedipalp, submarginal, composed of 10 stiff flat bacilli with blunt end. It is poorly developed, though easily seen and is not accompanied by small spines as in preceding species. End of lip and inner angles of maxillæ densely covered with granules. *Lip* wider than long in ratio 28:22, with almost parallel sides, curved at base, emarginated at end. An area of smooth chitin on sternum behind lip. Both the pro- and the retromarginal scopula of the cheliceræ, the maxillary scopula and the hair fringing the lip are red, while the hair covering the rest of the lip and the maxillæ is dark brown. *Sternum* longer than wide, clothed with short brown and long black hair. Four sigilla. First pair opposite second coxæ, second pair opposite third coxæ, much larger and long, separated from margin by almost their length. Their anterior ends separated from each other by twice the length of the sigilla. Palpi, first and second legs much heavier than third and fourth legs. Legs 1423. First leg—41 mm. long, second—38 mm., third—32 mm., fourth—41 mm. A velvety pad on retrolateral surface of fourth femur. All legs fringed with a lateral brush of hair on both sides of patella, tibia and metatarsus. A lateral brush on the retrolateral surface of first and second femora, and on both sides of third and fourth femora. Scopulæ very dense. Those of fourth tarsi bisected by a narrow line of setæ, those of all other tarsi entire. First and second metatarsi scopulate to base, third metatarsus in distal half, fourth metatarsus in distal fifth. First leg without spines. Its metatarsus short, though distinctly longer than tarsus. No spines on second leg. Two terminal spines below on third metatarsus, as well as on fourth metatarsus. The latter slightly longer than tibia. Two longitudinal broad lines bare of all hair on all patellæ. Legs clothed with short grey recumbent and long curved hair which is dark at base but becomes grey toward end. Claws entirely concealed by ungual tufts, strongly curved, smooth. Palp with a

heavy scopula on terminal joint. Color in alcohol reddish-brown above, abdomen brown, third and fourth legs yellowish-brown. Below, abdomen and third and fourth legs same color as above, rest darker, especially first and second pair of coxæ. Habitat: Panama (definite locality not given).

Family Barychelidæ

Merothele gen. nov.

Thoracic groove transverse. ALE larger than PLE. Laterals separated from each other by almost the length of PLE. PME almost round. Anterior row procurved, posterior recurved. Rastellum wanting. All tarsal scopulæ bisected longitudinally by setæ. Claws with teeth. Lip and maxillæ spinulose. Basal joint of spinnerets longer than the middle and terminal together, dorsally subdivided into two sclerites. This genus belongs in the sub-family Leptopelmateæ. Type **M. zebra.**

Merothele zebra sp. nov.

Female from Cerro Flores. Total length with cheliceræ 17 mm. Carapace 6.0 mm. long, 4.8 mm. wide, widely truncated in front. Thoracic groove transverse. Eyetubercle low, somewhat protruding beyond edge of carapace. Eyegroup transversely rectangular. ALE largest, PME smallest. Anterior row procurved, posterior row recurved. AME separated from each other by their radius and from ALE by more than their radius. PME almost round, equidistant from AME and PLE, the distance being the same as that between AME. Laterals separated from each other by almost the length of the PLE. Quadrangle much narrower in front than behind, not quite twice as wide as long. Eyearea behind posterior row clothed with black bristles directed forward; in front of first row with dark brown bristles directed backward. *Cheliceræ* without rastellum, but with heavy bristles curved downward. Promargin with a row of twelve black teeth, a few small tubercles at base between margins and a very scant promarginal scopula. Retromarginal scopula heavy, red. *Lip* very slightly wider than long, with spicules at tip. *Maxillæ* with spicules at inner angle, with heavy red scopula. *Sternum* as long

as wide, with black bristles and recumbent grey hair. Three pairs of sigilla, all submarginal and oval. All tarsi scopulate. Those of first and second pair bisected longitudinally by a narrow line of setæ, those of third and fourth pair by a wide band of setæ. First metatarsus scopulate on both sides to base. Second metatarsus scopulate to base on proventral edge, in apical third on retroventral edge. Third and fourth metatarsi scopulate on both sides near distal end. On dorsal surface of all tarsi between longer hairs and trichobothria several club-shaped short hairs or scales. Claws curved, with several teeth. *Legs* 4123, with spines and spinelike bristles. Anterior metatarsi with 1-1 ventral spines of which the distal one is apical. Second metatarsi same as first. Third and fourth metatarsi with three ventral apical spines and with several spinelike bristles and at least two true ventral spines near middle. All tibiæ with only spinelike bristles. Dorsal real spines are only on third and fourth leg, especially on third metatarsus. Lower spinnerets short, separated from each other by less than half their diameter. Posterior spinnerets when viewed from below appear three-jointed. Their terminal joint short, cone-shaped, together with median joint shorter than basal joint. When viewed from above posterior spinnerets appear four-jointed, the basal joint being divided into two segments by an oblique suture or interarticular membrane extending from middle of the joint on inside to base on outside. The interarticular membrane is here of the same appearance as elsewhere. Moreover, between the basal sclerite and the abdomen is a clearly visible interarticular membrane. Scopulæ of legs and palpi iridescent. Carapace, cheliceræ and femora dusky brown, rest of legs chestnut brown. Abdomen purplish-brown with a row of five median grey spots on back and five pairs of grey stripes running obliquely backward. Spinnerets dusky brown. Venter greyish-brown.

Family Dipluridæ

Accola spinosa sp. nov.

Pullus. Total length including cheliceræ 5.3 mm. Carapace oval, 1.95 mm. long, 1.51 mm. wide, quite flat. Eyegroup small, compact. Eyes 6. Anteriors subcontiguous, larger than and contiguous with PLE. PME smallest, round, slightly, but

distinctly separated from the other eyes. The eyes occupy the entire tubercle which is rather low and almost reaching anterior edge of carapace. *Chelicerœ* with a row of 8 or 9 small black teeth on promargin, with scopula on retromargin. *Lip* wider than long, its base semicircular. *Sternum* ovoid, slightly longer than wide. *Legs* 4132. Spines on all legs but especially numerous on third and fourth. Upper claws with several long teeth, lower claw with three teeth. Palpal claw with a row of about 20 long teeth. Thoracic groove small, procurved. General color in alcohol yellow, abdomen greyish-yellow. Hair brown and quite long, bristle-like. Fine recumbent long greyish hair also present on legs and abdomen. Habitat: Wilcox camp on San Lorenzo River.

Family Ctenizidæ

Ancylotrypa panamana sp. nov.

Female. Total with cheliceræ 15 mm. Carapace 6.0 mm. long, 4.6 mm. wide. Cephalic region high, twice as long as thoracic. Thoracic groove procurved. Anterior edge of carapace straight, carapace almost as wide in front as in middle. Clypeus equal to the diameter of AME. Eyegroup transversely rectangular, much wider than long. ALE largest, the other eyes sub-equal in size. Anterior row straight by anterior edges, posterior row slightly recurved. Anterior eyes almost equidistant, separated from each other by more than the radius of AME. PME separated from PLE by half of the radius of the AME, from ALE by the diameter of the AME. PME widely separated from each other. Laterals separated from each other by the radius of the AME. Quadrangle much narrower in front than behind, twice as wide as long. In front of eyegroup a few stiff curved bristles. *Chelicerœ* with rastellum, with only a retromarginal scopula. Promargin with 5 black teeth, first tooth being a compound of four teeth of which the proximal is very large. An area of numerous black tubercles between the teeth and the scopula. *Lip* free, longer than wide in ratio 32:24, ovoid, narrowed toward tip and covered with hair only. Base of lip semicircular. *Maxillæ* with scopula and a few tubercles near inner angle, almost as wide as long. *Sternum* ovoid, longer than wide in ratio 80:60. Three pairs of sigilla. First and

second pair long and narrow, third much larger, oval, separated from margin by their length. Pedipalpi with spines on tibia and terminal joint which is heavily scopulate and has a smooth claw. *Legs* 4123.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	4.100	2.460	2.993	2.583	1.763	13.899
II	3.690	2.378	2.583	2.132	1.312	12.095
III	2.952	1.886	1.435	1.845	1.353	9.471
IV	4.100	2.460	3.280	2.870	1.517	14.227

Upper claws of the first and second pair of legs with a double row of teeth, of the third and fourth pair with a single row. Third claw smooth. Numerous trichobothria on all tibiæ, metatarsi and tarsi. First and second tarsi with a scopula, first metatarsus scopulate in distal half. Legs, especially those of third pair, heavily spined. Prolateral surface of third patella covered with short heavy spines comparable to a rastellum and evidently used in digging, arranged in rows of 4-4-3-1. Other spines on legs: First leg, tibia—ventral 1-1 bristles, metatarsus—ventral 1-1-3, no spines elsewhere. Second leg, tibia—ventral 1-1 bristles, metatarsus—ventral 2-2-3, tarsus—ventral 1, no spines elsewhere. Third leg—patella—dorsal 0, prolateral 0, retrolateral 1-1, ventral 0. Tibia—dorsal 1, prolateral 1-1, retrolateral 1-1, ventral 1-2-2. Metatarsus—dorsal 1 near middle. prolateral 1-1-1-1-1 (or 6), retrolateral 1-1-1-1-1 (or 6), ventral 2-1p-4. Tarsus dorsal 0, prolateral 1-1-1-1-1-1-2, retrolateral 0, ventral 1 beyond middle. Fourth leg, femur—dorsal a group of spines at distal end comparable to a rastellum, tibia—ventral 1p-1p-1p-2 bristles, metatarsus ventral 3-3-1p-1p-1p-3, tarsus—ventral 1-1, elsewhere none. Legs clothed with simple black hair, abdomen with simple hair of variable length. Spinnerets four, the three joints of the posterior spinnerets about equal in length. Anterior spinnerets single-jointed, short. Color in alcohol: cheliceræ black, carapace blackish-brown, thoracic portion considerably lighter than cephalic. Abdomen grey. Femora dark grey, tibiæ and metatarsi yellow, tarsi dark. Lip darker than sternum which is yellow. Coxæ yellow. Spinnerets light yellow. Found in a shallow tunnel web under a log at the Wilcox camp on the San Lorenzo River.

SUB-ORDER ARACHNOMORPHÆ

FAMILY ACANTHOCTENIDÆ

Acanthoctenus maculatus sp. nov.

a) Immature specimen from Santiago. Total length 6.97 mm. Sex uncertain, presumably an immature female. Carapace 2.635 mm. long, 2.295 mm. wide, not very high and much narrowed in front. Thoracic groove longitudinal. Ratio of eyes AME : ALE : PME : PLE = 6 : 4.5 : 10 : 11. AME separated from each other by their radius. PME separated from each other by 4/10 of their diameter. PLE separated from each other by 28/11 of their diameter. Quadrangle wider behind than in front in ratio 22 : 15.5, wider than long in ratio 22 : 15. PLE nocturnal, separated from AME by 7/9 of their diameter and by twice that distance from AME. A straight line passing through the centers of PME is tangent to ALE. Clypeus equal to diameter of AME. On the clypeus a pair of bristles near the edge, followed by a transverse row of four bristles, then by a single bristle in line with the lower edge of AME, then a pair of small bristles in line with posterior edge of AME, then five bristles in a curved line. There is also on each side a bristle in front of PLE. *Cheliceræ* with yellow boss. Promargin with 3 teeth of which the median is strongest, retromargin with 3 equal and equidistant teeth. Scopula on promargin only. *Lip* wider than long in ratio 15 : 13, truncated at apex, with basal excavations; its end just reaches the middle of maxillæ. *Maxillæ* sub-parallel, with scopula and serrula. *Sternum* as wide as long, wide in front, pointed behind, but withal sub-rotund. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.635	3.995	2.380	0.935	9.945
II	2.635	3.230	2.125	0.850	8.840
III	2.295	2.295	1.955	0.935	7.480
IV	2.975	3.230	3.230	1.105	10.540

No scopulæ on tarsi. Trichobothria numerous and irregularly arranged on all tibiæ, metatarsi and tarsi. Ungual tufts well developed. Claws two, arched, with five teeth of which the proximal four are very small. Palpal claw with two teeth.

Spines on legs: First leg—Femur, dorsal 1-1-1 small spines,

prolateral 1-1 small spines, retrolateral 1 distal, ventral 1p-1p long spines and 1r short spine. Patella 0. Tibia, dorsal 0, prolateral 0, retrolateral 0, ventral 2-2-2-2-2-2-2-2-2 very long spines. Metatarsus, dorsal 0, prolateral 0, retrolateral 0, ventral 2-2-2-2-2 long spines. Second leg—same as first except: femur 1p-1p short spines and 1r short spine, tibia prolateral 1-1-1 small spines, ventral 9 pro- and only 7 retrospines. Third leg—Femur, dorsal 1-1-1, prolateral 1-1-1, retrolateral 1-1, ventral 0. Patella, only retrolateral 1. Tibia, dorsal 1-1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus, dorsal 1-1-1, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2. Fourth leg same as third. Calamistrum small, irregular, occupying only about basal eighth of fourth metatarsus. Spinnerets six. Cribellum divided. (In the younger specimen from the Wilcox camp the calamistrum is not yet developed and the cribellum but poorly defined.)

Color in alcohol: Carapace with a broad, median longitudinal yellow band limited anteriorly by the black discs surrounding the posterior four eyes, and laterally by a right and left black band. The latter is separated from the edge of the carapace by a narrow marginal yellow band which is mottled or speckled with black. Legs yellow, ringed with black (yellow without black rings in the younger specimen). Abdomen grey with a $\wedge$-mark in middle, with black spots here and there behind the mark, and with peculiar tufts of white hair. Venter yellowish-grey. Spinnerets with some black on outside.

b) Immature specimen from Ancon Hill, probably a male. Total with cheliceræ 6.912 mm. Carapace 3.034 mm. long, 2.542 mm. wide, narrowed in front to 1.435 mm. in the region of the second row of eyes. Posterior limit of head indicated by slight depression. Thoracic groove longitudinal, long, situated halfway between the posterior limit of the head and posterior edge of carapace. Generally speaking, carapace is rather flat. Posterior declivity begins from middle of thoracic groove. Ratio of eyes AME:ALE:PME:PLE = 6:4:9:9.5. AME separated from each other by 9/12 of their diameter and by their diameter from ALE. AME separated from PME by their radius. PME are separated from each other by 4/9 of their diameter, from PLE by 8/9 of their diameter, and from ALE by 3/9 of their

diameter. ALE are separated from PLE by 10/19 of the diameter of the latter. Quadrangle wider behind than in front in ratio 21 : 14.5, wider behind than long in ratio 21 : 17.5. Clypeus is equal to the diameter of the AME. Arrangement of eyes as in Ctenus. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	3.403	5.166	3.116	1.107	12.792
II	3.075	4.264	2.747	0.962	11.048
III	2.665	3.198	2.460	1.066	9.389
IV	3.608	4.223	4.018	1.476	13.325

Spines: First leg—Femur, dorsal 1-1-1, prolateral 1-1, retrolateral 1-1-1-1, ventral 2-2-2-2-2 of which first pair begins in middle and the first and second prospines are the only ones of exceptional length reaching to base of third and fourth spine respectively. Patella 0. Tibia, dorsal 0, prolateral 1-1-1-1 small spines, retrolateral 1-1, ventral 2-2-2-2-2-2-2-2-2 very long spines of which the fifth pair is the longest reaching beyond base of 8th pair, while 9th pair is shortest. Metatarsus dorsal 0, prolateral 1 near base, retrolateral 0, ventral 2-2-2-2-2 very long spines, third pair being the longest. Second leg same as first, except: Femur prolateral 1-1-1-1-1, ventral 0. Tibia prolateral 1-1. Metatarsus retrolateral 1-1, ventral 2-1p-2-2-2-1p-2-2-2 (*i. e.*, 9 pro- and only 7 retroventral spines. The latter are considerably longer than the proventral ones, especially the second and third, reaching beyond base of 6th and 7th spine respectively). Third leg, Femur—dorsal 1-1-1, prolateral, 1-1-1-1, retrolateral 1-1-1-1, ventral 0. Patella dorsal 1 small apical bristle, prolateral 1, retrolateral 1, ventral 0. Tibia dorsal 1-1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus dorsal 0, prolateral 1-2-2, retrolateral 1-1-2, ventral 2-2-2. Fourth leg same as third except: femur prolateral 0-1-1-1, retrolateral 0-1-1. Metatarsus prolateral 1-1-2, retrolateral 1-2-2. Palp—Femur dorsal 1-1-1, prolateral 0-2, retrolateral 0, ventral 0. Patella dorsal 1-1, prolateral 1, retrolateral 0, ventralo. Tibia dorsal 1-1, prolateral 2 near base, retrolateral 0, ventral 0. Terminal joint dorsal 0, prolateral 2-1, retrolateral 1, ventral 0. All trochanters notched. Hair on legs simple. Trichobothria numerous on tibiæ, metatarsi and tarsi, apparently without order. Claws two.

FAMILY OONOPIDÆ

Oonops reticulatus sp. nov.

Female. Total 1.94 mm. Carapace 0.68 mm. long, 0.50 mm. wide, considerably narrowed in front, yet wider than eyegroup. Thoracic groove wanting. Carapace high, level in middle third, sloping down gently anteriorly and steeply posteriorly, shiny with sparse hair in median line. Eyes subequal in size. Anterior pair touching edge of carapace so that a clypeus is wanting. Anterior pair is separated from each other by a little less than their length, subcontiguous with PME and PLE. Second row recurved, PME contiguous and sub-contiguous with PLE. All six eyes in a black setting following outline of eyes so that there remains a yellow area in front of PME between anterior eyes. *Chelicerœ* weak, cone-shaped, not armed. *Lip* free, wider than long, trapeze-shaped, emarginate at tip. *Maxillœ* strongly inclined over lip. *Sternum* oval, wide in front, longer than wide in ratio 12:10. An interesting feature of the sternum is the presence of three pairs of chitinized grooves intercoxal in position, parallel to each other and at right angles to margin, analogous to the sigilla of Theraphosid spiders. (A similar case is described later in *Clubiona sigillata.*) Palp without claw. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	0.576	0.625	0.396	0.288	1.885
II	0.504	0.576	0.360	0.288	1.728
III	0.432	0.522	0.324	0.252	1.530
IV	0.612	0.720	0.504	0.288	2.124

No true spines on legs. Claws strongly curved, with 2 teeth. One trichobothrium on each tibia, two on each metatarsus. Hair on legs, when examined under high power, shows minute spines. Color in alcohol: Carapace, cheliceræ, legs, lip, sternum, maxillæ and spinnerets yellow. Abdomen purplish-grey with a reticulum of irregular polygons. Venter light grey. Habitat: Panama City.

FAMILY THERIDIIDÆ

Argyrodes cingulatus sp. nov. (Figures 3, 4 and 5).

Female. Total including spinnerets 3.075 mm. Carapace 1.353 mm. long, 0.861 mm. wide, widest in middle, narrower in

front than behind, rounded anteriorly, emarginate behind. Thoracic groove shallow, transverse, almost straight, almost half as long as greatest width of carapace, situated a little more than two thirds behind anterior edge. AME on a rectangular tubercle, laterals on each side on a common small tubercle. Anterior row strongly recurved, posterior row lightly recurved so that a straight line passing through centers of PLE is tangent to PME.

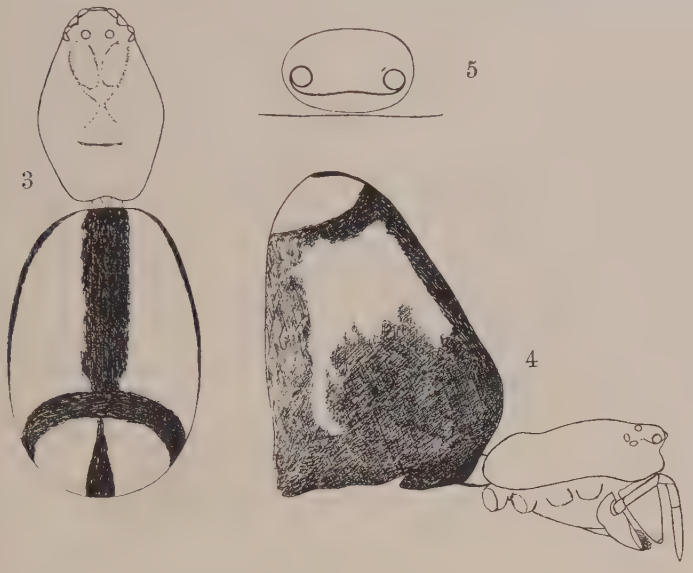


FIGURE 3, **Argyrodes cingulatus** sp. nov., dorsal view. FIGURE 4, Same, side view. FIGURE 5, Same epigynum.

Ratio of eyes AME : ALE : PME : PLE = 3 : 2 : 2 : 2. AME separated from each other by their diameter, from ALE by 2/3 of their diameter or the diameter of the ALE, from PME by 2.5/3 of their diameter. PME separated from each other by the same distance as from AME and also by the same distance from ALE. Laterals sub-contiguous. Quadrangle wider in front than behind in ratio 7 : 6, as long as wide behind. Clypeus in longitudinal section at first concave, then convex, not quite as high as quadrangle (ratio 5 : 6). Femora except those of third pair curved downward. Metatarsi, especially fourth, much thicker at base than at end. Tibiæ much thicker toward both ends

than in middle, first tibia slightly curved. Spines and trichobothria wanting, but a typical "drum" in middle of each tarsus. Upper claws with 2 or 3 small teeth, distal one largest. Third claw smooth, longer than upper claws and curved at end in a direction opposite to that of the principal curvature. Palpal claw almost straight, long and thin, with a single small tooth. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.501	2.378	1.968	0.943	7.790
II	1.558	1.394	1.107	0.615	4.674
III	0.820	0.656	0.574	0.369	2.419
IV	1.312	1.025	0.861	0.492	3.690

Coloration resembles somewhat that of *A. jucundus* and the epigynum that of *A. argyrodes*. Carapace dark brown with indistinct black lines as in Figure 3. Abdomen best understood from Figures 3 and 4 showing it from above and in profile. A broad black median band extends from anterior border to the black ring which encircles the rounded end of the dorsum. The end itself is bisected longitudinally by a black band extending only half way and much narrower in front than behind. There are two large, irregular lateral silver fields, a large median silver field on the posterodorsal declivity of the abdomen, and an hemispherical apical field partly divided by the black band mentioned above, all silver fields represented white in the figure. Epigynum large, prominent, transversely oval, brown, with two widely separated round receptacles (Figure 5). Legs dark, except metatarsi and tarsi which are yellow. Base and end of first and second metatarsi dark. Palpi also dark. Sternum, lip and maxillæ dark, the latter with yellow inner edge, lip with yellow tip. Behind epigynum a square silver spot ending posteriorly in a transverse silver line. Sternum glabrous, of the typical triangular shape, widest between coxæ of first pair, pointed behind between fourth coxæ which are slightly separate. It is much longer than wide in ratio 18:14. Lip much wider than long in ratio 7:3.5. Maxillæ with parallel truncatures covered with scopula. Serrula long. Margins of cheliceræ short, transverse. Promargin with 3 teeth, median smallest, retromargin with 1 tooth. Scopulæ wanting. Basal joints of cheliceræ divergent. Habitat: Panama City.

Dipoena pulchella sp. nov. (Figures 6-9).

Female. Total 2.050 mm. Carapace 0.851 mm. long, 0.740 mm. wide. Abdomen ovoid, 1.558 mm. long, 1.148 mm. wide, considerably overlapping carapace, the petiolus being in the anterior part of the middle third, the epigynum about in middle and the genital fold three-fifths from anterior end (Figure 6). Stridulating organs wanting. Carapace very high, measuring fully 0.481 mm. in the region immediately behind PME, both the posterior

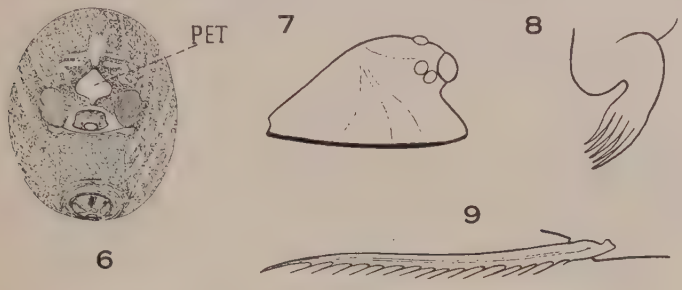


FIGURE 6, **Dipoena pulchella** sp. nov., ventral view of abdomen, showing the petiolus immediately in front of the epigynum. FIGURE 7, Same, side view of carapace. FIGURE 8, Same, palpal claw. FIGURE 9, Same, bristle of the fourth tarsus.

declivity and the clypeus forming angles of about 45° and the head projecting over the clypeus. Edge of carapace rebordered. Eyes in two rows. Viewed from above anterior row appears recurved, posterior straight. Viewed from in front anterior row is slightly downcurved, posterior row strongly downcurved. AME only diurnal, largest and wider apart from each other than from ALE. Ratio of eyes AME : ALE : PME : PLE = 3.2 : 2 : 2.3 : 2. AME separated from each other by 2/3.2 of their diameter, subcontiguous with ALE and as far removed from PME as from each other. PME separated from each other by their radius, from PLE by their diameter. Laterals sub-contiguous, separated only by a black line. Quadrangle wider in front than behind in ratio 7.5 : 5 longer than wide behind in ratio 7 : 5. Clypeus concave in median plane, convex transversely, almost as high as two diameters of AME. There is no hair on the carapace. *Chelicerœ* weak and shorter than clypeus, completely concealed below by the maxillæ. Fang exceedingly small, margins practically wanting,

since basal joint is cone-shaped, the inner edge of the cone being covered with a row of stiff black hairs resembling spines. *Lip* almost triangular and as long as wide at base. *Maxillæ* arched over lip, meeting with their ends, but the arch is formed by the inner edge of the maxillæ only, while the truncatures form an angle between them open anteriorly. *Sternum* longer than wide in ratio 18:13, evenly rounded in front, produced behind between the fourth coxæ which are separated from each other by their diameter. The convexity of the sternum increases posteriorly. Only a few scattered hairs on sternum. *Legs* stout and short. Metatarsi and tarsi distinctly cone-shaped. No spines on legs, but hair is stiff. A row of 4 or 5 trichobothria on tibiæ, and a single trichobothrium on each metatarsus beyond middle. Fourth tarsi with nothing but serrated bristles, with long teeth. The bristles which form an inconspicuous comb are only six in number. There are also serrated bristles on the fourth metatarsus. Upper claws of first leg very peculiarly structured inasmuch as the end is forked and the edge of the base minutely toothed on one side. The claws of the fourth leg are simple, with a retrorow of indentations. Palpal claw is quite unusual in appearance, resembling a fork with five equal teeth (Figure 8). Patella and tibia of palp heavier than femur, terminal joint cone-shaped. Tibia only one and one half times as long as wide. There is one trichobothrium at the end of first third of tibia of palp. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	0.740	0.814	0.481	0.296	2.331
II	0.629	0.666	0.444	0.296	2.035
III	0.629	0.629	0.444	0.296	1.998
IV	0.814	0.851	0.592	0.310	2.567

Spinnerets surrounded by a fold of the abdominal wall forming not a quite perfect hexagon. Anterior spinnerets much heavier and somewhat longer than posterior pair. Medians minute. Colulus wanting. Anal tubercle large, tranversely oval. Epigynum small, with a transversely oval opening and two minute receptacles. Color in alcohol: Carapace dusky olivaceous brown with dark marginal line. Legs same as carapace except trochanters and proximal half of 4th femur which are yellow. Sternum, lip and maxillæ of the same color as carapace. Abdomen below dark greyish-brown with two parallel greyish-yellow lines extend-

ing to circummammillary wall. Sides with oblique greyish-yellow stripes, three of which extend in concentric arcs behind the spinnerets. Lungs greyish-yellow. Dorsum of abdomen dark, minutely dotted with greyish-yellow and with four parallel fine lines running from one round spot to another. Hair on abdomen fairly long. Habitat: Wilcox camp on San Lorenzo River.

Latrodectus mactans (Fabricius) (Figures 10-12).

Our figures represent the coloration of the abdomen of a female from Las Palmas. This common American species is

10 11 12

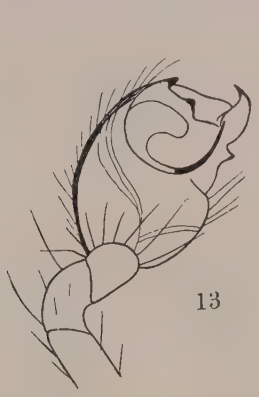
FIGURE 10, *Latrodectus mactans* (Fabricius), female, abdomen in side view. FIGURE 11, Same, dorsal view. FIGURE 12, Same, ventral view.

exceedingly variable as to coloration, especially in the female. I reserve for a future occasion a comparison of specimens from different localities.

Theridion defunctum sp. nov. (Figures 13 and 14).

Male. Total without cheliceræ 1.722 mm. Carapace 0.703 mm. long, 0.666 mm. wide, low, glabrous, with just a few hairs behind and between the eyes. Thoracic groove a shallow transverse depression. Eyes in two rows. Anterior row recurved, posterior straight. Laterals contiguous. AME alone diurnal. All eyes of about the same size and nearly equidistant, but anteriors closer together than posteriors so that posterior row is somewhat longer than anterior. Quadrangle as long as wide behind. PME separated from each other by more than radius, but less than diameter. AME separated from each other by their radius. Clypeus equal to one and one-half diameters of AME and has two short horns directed forward. *Cheliceræ* narrow and long, as long as the width of the carapace. Fang weak, margins straight and short.

Teeth, scopulæ, boss and stridulating ridges wanting. *Lip* wider than long in ratio 4 : 3, much narrower at end than at base, and so thin that articulation of rostrum is clearly visible through the lip and is easily mistaken for a transverse line. The lip does



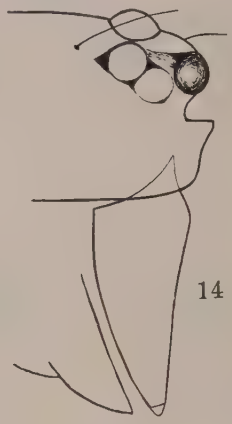


FIGURE 13, **Theridion defunctum** sp. nov., right palp from outside. FIGURE 14, Same, side view of anterior end of carapace.

not reach middle of maxillæ. *Maxillæ* inclined over lip, long, pointed at inner apical angle. Scopula very poor, but serrula long, extending to outer edge of maxilla. *Sternum* convex, triangular, as wide as long, widest anteriorly, with but a few erect hairs, shiny. Fourth coxæ separated by the end of the sternum which merges with the posterior margin of the carapace, right and left of the petiolus. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	1.443	1.665	1.221	0.555	4.884
II	0.888	0.999	0.666	0.407	2.960
III	0.518	0.555	0.370	0.296	1.739
IV	0.888	0.851	0.629	0.370	2.738

Legs clothed with two kinds of hair, both kinds forming regular rows. The long hair forms an angle of 60°, the short hair, which is no longer than one third or one half of the long variety, forms an angle of only 20° with the axis of the leg. Upper claws dissimilar, retroclaw with 4 teeth of which the distal one is long, while the other three are quite short; proclaw with but a single

small tooth. Third claw long, smooth, curved, forming more than a semicircle. Trichobothria absent except for a single one on each tibia a little in advance of middle. Bristles of comb on fourth tarsus not distinct from other bristles except when viewed under high power. Each tarsus has a "drum" before middle. Spines few and slender, not always distinguishable from hair. On first and second leg only on patella dorsal 1-1 and on tibia dorsal 1-0-1. On third and fourth leg besides these spines also a ventral distal spine on femur. Abdomen egg-shaped, narrower in front than behind. Genital groove behind middle, genital area in front of groove glabrous, extending to anterior end of abdomen and clearly delimited. Tracheal spiracle close to spinnerets Colulus wanting. Anterior spinnerets contiguous. Posterior spinnerets slightly shorter than anteriors. Abdomen sparsely clothed with long hair. Stridulating organ wanting. Bulb of pedipalp as in Figure 13. Embolus begins with an enlargement near distal end of bulb, runs downward and then forward in a curve. Tibia of palp short, without apophysis. Color in alcohol: Generally yellow with bulb darker. On carapace a median dark band composed of minute dots extends to thoracic groove. Edge of carapace with a narrow black line which is not clearly defined. Abdomen dorsally with an anterior median dark spot followed by two rows of three dark spots each and smaller spots in between. Sides of abdomen with small white spots. Edge of sternum with narrow black line except in front. Habitat: Wilcox camp on San Lorenzo River.

Family Linyphiidæ

Gongylidiellum panamanum sp. nov. (Figures 15-17).

Female. Total 1.517 mm. Carapace 0.615 mm. long, 0.533 mm. wide, considerably narrowed in front, so that eyes when viewed from above occupy entire width. Thoracic groove longitudinal. Eyegroup on a square elevation. Eyes approximately of the same size except AME which are smaller. Anterior row recurved, posterior straight. Posterior eyes equidistant, separated by their radius. Laterals contiguous. Quadrangle as long as wide, slightly narrower in front. Anteriors equidistant, separated from each other by the radius of AME. Clypeus as high as diameter of AME. *Legs* 1423, thin, with very few spines.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	0.666	0.740	0.629	0.444	2.479
II	0.592	0.740	0.518	0.407	2.257
III	0.481	0.555	0.444	0.333	1.813
IV	0.666	0.740	0.555	0.407	2.368

Several trichobothria on tibiæ, distal one longest. No trichobothria elsewhere. Upper claws smooth, third claw strongly curved, with one small tooth. *Cheliceræ* stout, without boss or stridulating ridges. Margins oblique. Promargin with three teeth, retromargin with three much smaller teeth. *Lip* wider than long, strongly rebordered, not reaching middle of maxillæ.

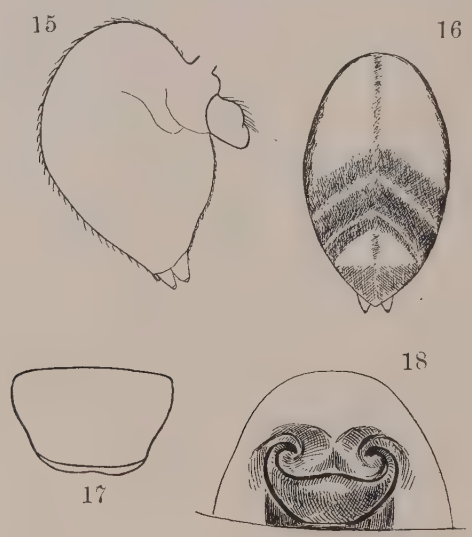


FIGURE 15, **Gongylidiellum panamanum** sp. nov., side view of abdomen. FIGURE 16, Same, dorsal view of abdomen. FIGURE 17, Same, epigynum. FIGURE 18, *Linyphia calcarifera* (Keyserling), epigynum.

Maxillæ slightly divergent, angular at end. *Sternum* convex, triangular, as wide as long, pointed between fourth coxæ which are separate. First coxæ wide apart. Surface of sternum glabrous with a few rough granules. *Abdomen* soft. By far the most conspicuous organ is the red-brown epigynum shown in Figures 15 and 17. It is convex, wider than long, strongly pro-

truding. Color in alcohol: Carapace and legs dark yellow with an olivaceous tinge. Ends of coxæ below with a black marginal line. Sternum dark olivaceous. Abdomen dark olivaceous and grey, the dark forming a pattern represented in Figure 16. Spinnerets olivaceous. Habitat: Wilcox camp on San Lorenzo River.

Linyphia calcarifera (Keyserling) (Figure 18).

The specimen which Keyserling described was an immature one. The specimen described here is a mature female which undoubtedly belongs to this species. A detailed description and a figure of the epigynum are therefore not out of place.

Female. Total without cheliceræ 3.744 mm. Carapace 1.353 mm. long, 0.820 mm. wide, as wide behind as in front. Anterior edge of carapace rounded, posterior slightly recurved. Thoracic groove in the shape of a rather wide longitudinal depression. Surface of carapace distinctly reticulated and devoid of hair. Eyes in two rows of equal length, PME considerably larger than all others. Laterals separated only by a thin black line, and equal in size, situated on a joint tubercle. Viewed from above anterior row is recurved, posterior row straight. Viewed from in front the anterior row is straight, the posterior row curved downward. Ratio of eyes AME: ALE: PME: PLE = 1.5: 2: 3:2. AME separated from each other by not quite their diameter, from ALE by twice their diameter. PME separated from each other by 4/3 of their diameter, from PLE by 1.5/3 of their diameter (= by their radius). Quadrangle much narrower in front than behind in ratio 4.5: 9, wider than long in ratio 9: 7. PME on a joint tubercle. *Clypeus* as high as 6/7 of the length of the quadrangle. *Cheliceræ* parallel by outer edges. Margins strongly oblique. Promargin with 4 teeth of which second proximal is longest, third almost as long, while first and fourth are much smaller. Retromargin with 5 small teeth. Boss wanting. Stridulating ridge barely noticeable. Scopulæ wanting. *Lip* wider than long with short parallel sides, procurved base and recurved end, strongly rebordered, does not reach middle of maxillæ. *Maxillæ* wider at end than at base, slightly divergent, but their outer edge parallel to their inner edge and forming an angle with anterior edge. Scopula occupies entire truncature (inner edge). Serrula long, reaching beyond outer angle and extending to outer edge.

Sternum triangular, longer than wide in ratio 22 : 16, produced between hind coxae which are separate, while first coxæ are wide apart. Surface of sternum distinctly retriculated and devoid of hair. *Legs* 1423.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	1.517	1.681	1.435	0.738	5.361
II	1.394	1.517	1.230	0.656	4.797
III	1.025	1.025	0.902	0.451	3.403
IV	1.640	1.476	1.435	0.656	5.207

Spines. First and fourth femora with a basal midventral spine, second and third femora with 1-1 midventral spines in proximal half. All patellæ with 1-1 middorsal spines. First and second tibiæ with 1-1 dorsal, 1 prolateral, 1 retrolateral and 2-2 ventral spines, the latter not forming real pairs. Third tibia with 1-1 dorsal, 1 prolateral, 1 retrolateral and no ventral spines. Fourth tibia only with 1-1 dorsal spines. First and second metatarsi with 1 dorsal spine at base, 1 prolateral, 1 retrolateral in proximal quarter and no ventral spines. Third metatarsus with 1 dorsal spine at base, 1 prolateral, no retrolateral and 1-2 ventral spines. Fourth metatarsus only with 1 dorsal spine at base and 1 prolateral spine. Palpi with many long spines. Trichobothria completely wanting. Upper claws very little curved, with only two small teeth. Third claw strongly curved, with one tooth. Two pairs of serrated bristles or spurious claws. Palpal claw smooth. Abdomen constricted in middle, produced behind the spinnerets. Tracheal spiracle immediately in front of spinnerets. Colulus long. Epigynum shown in Figure 18. When viewed from a certain angle, it has the appearance of a cup. Color in alcohol: Carapace, cheliceræ, lip, maxillæ and sternum dark brown; maxillæ and lip with yellow edge. Palpi and fourth coxæ dark. Legs yellow. Abdomen above and below dark grey-brown with a transverse white band in middle, which does not extend over ventral surface, and oblique lateral white spot in front of transverse band on each side and a longitudinal lateral band on each side behind the transverse band. The ends of these lateral bands when viewed from below appear as two white spots on the ventral surface of the posterior projection of the abdomen, *i. e.* on the posterodorsal declivity of the abdomen. Habitat: Colombia and Panama.

FAMILY ARGIOPIDÆ

Meta gravida (Cambridge) (Figures 19-21).

(Although the description and figures given by Cambridge are quite sufficient for identification, they are too incomplete for careful comparison with specimens from other localities. Moreover Cambridge describes only the female and gives no exact measurements. The species is very variable both as to size and coloration. Some specimens have dark legs, others comparatively light

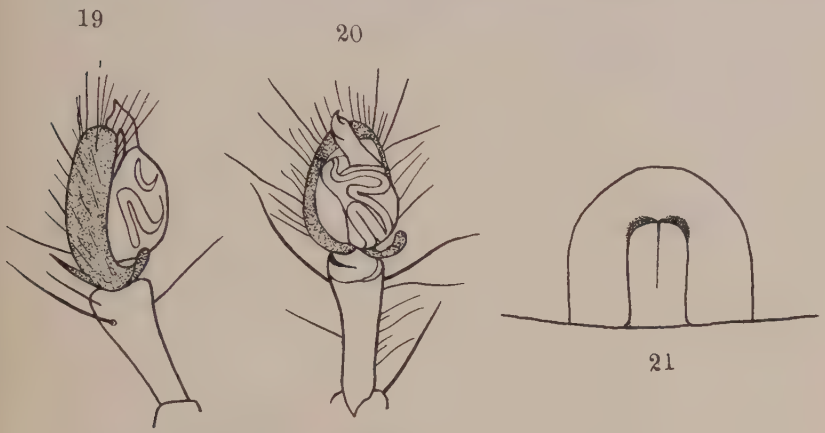


FIGURE 19, *Meta gravida* (Cambridge), right palp in side view. FIGURE 20, Same, from below. FIGURE 21, Same, epigynum.

with dark rings and spots. The abdomen is also subject to variation, but the epigynum is very much the same in all specimens and similar to that figured by Cambridge. The following detailed description of both sexes seems to be not out of place.)

a) Female. Total with chelicerae 10.08 mm. Carapace 3.840 mm. long, 3.168 mm. wide, but only 1.824 mm. wide in the region of the second row of eyes, which is not as wide as the carapace here. Thoracic depression semilunar, convexity of arc directed anteriorly. Surface of carapace clothed with fine hair. In middorsal line there are a few somewhat heavier hairs forming a row and ending in a pair in front of thoracic depression. A pair of similar hairs in middle of quadrangle, one hair on each side between PME and PLE and one hair behind each PLE. These hairs are occupying the same places as the bristles in other species. Eyerows of equal length. Anterior row recurved, posterior row

straight. Laterals contiguous, on a joint tubercle. Ratio of eyes AME : ALE : PME : PLE = 8 : 7 : 6 : 7. AME separated from each other by 3/8 of their diameter, from ALE by 6/8 of their diameter and from PME by 5/8 of their diameter. PME separated from each other by their diameter and from PLE by 7/6 of their diameter. Quadrangle as long as wide behind, wider behind than in front in ratio 18 : 16. Clypeus 6/8 of the diameter of AME. *Chelicerœ* powerful, basal joint 1.845 mm. long. Margins oblique. Promargin with three teeth, retromargin with four teeth. There is a distinct retromarginal scopula extending to the base of the joint. Promargin only with a few bristles. A row of four bristles on prolateral surface. Dorsal surface gibbous. Rudimentary boss present. Fang powerful. *Lip* wider than long in ratio 18 : 15, strongly rebordered and slightly emarginate at tip, just reaching middle of maxillæ. *Maxillæ* wide at end, diverging. Truncature short, but covered with a very thick scopula. Serrula very long, black. Outer angle sharp. *Sternum* distinctly heart-shaped, with a margin of smooth dark chitin which extends posteriorly between hind coxæ as a narrow strip all the way to the petiolus. If measured only to the end of the brown shield, sternum is longer than wide in ratio 50 : 44. Anterior edge emarginate. Sternum clothed with short black and long, erect, black hair. There is also a transverse row of some 8 or 10 long black bristles, extending in an almost straight line across the entire sternum between the first and second coxæ. All trochanters distinctly notched. Legs clothed with three different kinds of hair: (1) very short, black, forming an angle of 35° to the axis of the leg; (2) hair about five times longer than the first kind, but not nearly as numerous forming same angle with the axis of the leg; (3) long, erect hair. Trichobothria difficult to distinguish among the hair. Upper claws with a few small teeth. Third claw smooth. Six spurious claws. Small "drum" on all tarsi. Palpal claw with a few small teeth. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	9.216	9.600	10.560	1.722	31.098
II	5.280	5.760	5.760	1.230	18.030
III	2.496	2.496	2.304	0.779	8.075
IV	4.512	4.032	4.224	0.902	13.670
Palp	1.353	1.353	1.435		4.141

Spines. First leg. Femur—dorsal 1 near base, prolateral 1-1-1-1, retrolateral 1-1-1-1, ventral 0. Patella—dorsal 1-1, elsewhere 0. Tibia—dorsal 1-1, prolateral 1-1-1, retrolateral 1-1-1, ventral 0. Metatarsus—dorsal 1 near base, prolateral 1 near base, retrolateral 1 near base, ventral 0. *Second leg.* Same as first, except: Femur prolateral 1-1-1, retrolateral 0-1-1. Tibia—prolateral 1-1, retrolateral 0-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 0-1, retrolateral 0-1, ventral 0. Patella—dorsal 1-1, elsewhere 0. Tibia—dorsal 1 near base, prolateral 1 beyond middle, retrolateral 1 beyond middle, ventral 0. Metatarsus—dorsal 1 near base, prolateral 1 beyond middle, retrolateral 1-1, ventral 0. *Fourth leg.* Same as third, except: Femur—dorsal 1-1-1-1-1. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1. *Palp* has bristles in place of spines distributed as follows: Patella—dorsal 1-1, elsewhere 0. Tibia—dorsal 0-1, prolateral 1, retrolateral 1, ventral 0. Terminal joint dorsal 1, prolateral 1-1, retrolateral 1-1, ventral 1-1-1. *Abdomen* egg-shaped, wider end forward, overlapping carapace. Epigynum flat and small, with opening in the shape of a longitudinal rectangle. Spinnerets typical of the genus. Colulus small. Color in alcohol: Carapace yellow with head and sides darker. Legs reddish-brown. Palpi yellow. Cheliceræ yellow in front. Abdomen above greyish-yellow with a central heart-line and white spots of irregular outline all over. Below, cheliceræ, maxillæ, lip and sternum reddish-brown; coxæ and trochanters yellow; legs with indistinct dark markings at both ends of every joint. Venter of abdomen black from genital fold to spinnerets, bordered by a white iridescent band on each side. Anterior half of this band is straight, posterior incurved. Spinnerets dark brown. A row of three white spots on each side of abdomen.

b) *Male.* Total with cheliceræ 7.20 mm. Carapace 3.36 mm. long, 2.88 mm. wide, only 1.85 mm. wide in the region of the eyes. Abdomen 4.608 mm. long, 2.400 mm. wide. Legs 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	12.480	12.480	14.400	1.920	41.280
II	7.008	6.720	7.008	1.344	22.080
III	2.880	2.688	2.784	0.768	9.120
IV	4.896	4.704	4.704	0.864	15.168
Palp	1.394	1.189	1.230		3.813

Spines. First leg. Femur—dorsal 1 near base, prolateral 1-1-1-1, retrolateral 1-1-1-1, ventral 0. Patella—dorsal 0-1, elsewhere 0. Tibia—dorsal 1-1, prolateral 1-1-1-1, retrolateral 1-1-1-1, ventral 0. Metatarsus—dorsal 1 at base, prolateral 1 at base, retrolateral 1 at base, ventral 0. *Second leg* same as first, except: Femur—prolateral 1-1-1, retrolateral 1-1-1. Patella—dorsal 1-1 (the first a bristle, the second a spine). Tibia prolateral 1-1-1, retrolateral 0-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 0, ventral 0. Patella—dorsal 1-1 (the first a bristle, the second a spine), elsewhere 0. Tibia—dorsal 1 near base, prolateral 1, retrolateral 0, ventral 0. Metatarsus—dorsal 1 near base, prolateral 1-1, retrolateral 0, ventral 0. *Fourth leg* same as third, except: Femur—dorsal 1-1-1-1-1, prolateral 0-1 near distal end, retrolateral 0-1 near distal end. Tibia—dorsal 1-1, prolateral 1-1-1, retrolateral 0-1. Palp has bristles in place of spines, distributed as follows: Femur 0. Patella—dorsal 1-1, elsewhere 0. Tibia—dorsal 0-1, prolateral 1, retrolateral 1, ventral 0. Terminal joint—several bristles on cymbium.

Carapace with a deep, longitudinal thoracic groove ending posteriorly in a depression of the same type as in female. Eye-rows considerably shorter than width of carapace in that region. Ratio of eyes AME : ALE : PME : PLE = 6 : 6 : 6 : 6, *i. e.* all eyes are of the same size. Laterals on tubercle, distinctly but narrowly separated by about 1/6 of their diameter. AME separated from each other by 4.5/6 of their diameter, from ALE by 7.5/6 of their diameter, from PME by 5/6 of their diameter. PME separated from each other by their diameter, from PLE by 7/6 of their diameter. Quadrangle wider than long in ratio 18 : 16, wider behind than in front in ratio 18 : 16. Clypeus equal to diameter of AME. Anterior metatarsi flexible, twice as thin in middle as tibia in middle. Cymbium of palp with a basal apophysis directed outward and upward and ending bluntly, and with a spur at base on the dorsal surface. Copulatory apparatus projecting beyond cymbium. Color in alcohol much as in female, but pattern on abdomen clearer defined. Sides with a row of 5 confluent spots of light color, forming a longitudinal band and speckled with iridescent white. On dorsum, in posterior half two rows of 4 black spots each. White iridescent spots arranged in two logitudinal bands extend full length of abdomen. Habitat: Guatemala and Panama.

Bertrana hieroglyphica sp. nov. (Figures 22-28).

Male. Total 3.0625 mm. Carapace 1.480 mm. long, 1.184 mm. wide with a transverse thoracic depression as in *Araneus,* rather flat. A pair of bristles in center of quadrangle, one bristle on each side behind AME, one between PME and PLE, and one behind PLE. Also a band of bristles extending from PME to thoracic depression, the band consisting of two not quite regular rows. Both

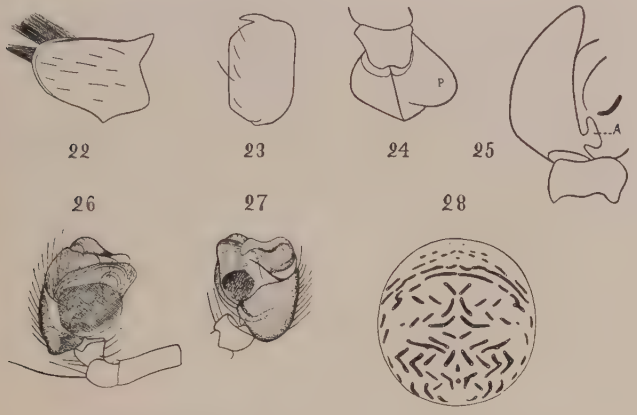


FIGURE 22, **Bertrana hieroglyphica** sp. nov., left maxilla from below. FIGURE 23, Same, left first coxa from below. FIGURE 24, Same, prolateral view of left third coxa. FIGURE 25, Same, right cymbium with apophysis. FIGURE 26, Same, right palp from outside. FIGURE 27, Same, right palp from inside. FIGURE 28, Same, dorsal view of abdomen.

rows of eyes recurved, anterior more so than posterior. Ratio of eyes AME:ALE:PME:PLE = 3.5:3:3:3. AME separated from each other by their diameter, from ALE by 3/3.5 of their diameter and from PME by 3/3.5 of their diameter. Laterals contiguous. PME separated from each other by their radius and from PLE by 5/3 of their diameter. Quadrangle wider in front than behind in ratio 10:7, longer than wide in ratio 8:7. Only AME are diurnal. Clypeus is equal to the diameter of AME. Basal joint of *cheliceræ* 0.703 mm. long. Boss rudimentary. Stridulating ridges wanting. Margins oblique, very poorly developed. One tooth is at the juncture of the two margins so that it is impossible to say with certainty to which margin it belongs, but

presumably to the promargin. This tooth is succeeded on the promargin by two teeth, on the retromargin by a row of 6 teeth of which the two terminal ones are large, while the other four quite small. There are besides two such small teeth between the two margins. Marginal scopulæ wanting. *Lip* wider than long, rounded, rebordered. *Maxillæ* wider at end than at base. Scopula extends to upper edge, is succeeded by a long serrula which ends at the base of a cone-shaped projection directed outward and upward (Figure 22). Patella of palp with two apical bristles of which the one on the prodorsal side is heavy, the other on the retrodorsal side much more slender. *Sternum* triangular, as long as wide, truncated in front, rounded behind. Posterior coxæ sub-contiguous, anterior pair widest apart. Sternum sparsely clothed with black, erect hair. No trichobothria on femora. On all tibiæ two rows of trichobothria. On all metatarsi a single trichobothrium before middle. None on tarsi. First coxa with a well-developed apical hook (Figure 23) directed forward. All coxæ, but especially those of third and fourth pair with a basal ventral projection (Figure 24, P) giving them a peculiar shape when viewed from the side. Trochanters not notched. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	1.640	2.050	1.230	0.492	5.312
II	1.394	1.394	1.025	0.410	4.223
III	0.820	0.902	0.574	0.328	2.624
IV	1.230	1.230	0.861	0.369	3.690

Spines. First leg. Femur—prolateral 1 beyond middle, elsewhere 0. Patella—dorsal 1-1 of which the first is very slender, the second prolateral in position to the black median stripe. Retrolateral 1 fine hairlike spine at end of protuberance, elsewhere 0. Tibia—dorsal 1-1 of which the first is retrolateral, the second prolateral to the black middorsal line; prolateral 1, retrolateral 0, ventral 0. Metatarsus—ventral 2 short spines near base, elsewhere 0. *Second leg* as first, except: Femur—dorsal 1 or 1-1, prolateral 1 a little before middle. Tibia—prolateral 1 a little before middle, ventral 2-1p-1p. Metatarsus 0. *Third leg.* Femur—dorsal 1 in middle, elsewhere 0. Patella—dorsal 1-1, retrolateral 1 slender spine at end of protuberance, elsewhere 0. Tibia—dorsal 1-1, prolateral 0, retrolateral 0, ventral 1 in middle. Metatarsus—dorsal 1-1, elsewhere 0. *Fourth leg* same as third,

except: Tibia—dorsal 1-0, prolateral 1 in middle. Metatarsus 1 in middle. *Abdomen* globular, higher than long and considerably overlapping carapace. Spinnerets typical. Colulus well developed. Anterior spinnerets heavier than the posterior ones, both two-jointed. Medians much smaller. Abdomen sparsely clothed with light-colored, erect hair, each hair sitting in a yellow dot at the end of the black bars. Palp as figured. Cymbium with a short basal retrolateral apophysis (Figure 26). Embolus long and thin, directed downward and outward (Figure 27). Color in alcohol: Generally greenish-yellow. Eyes surrounded by narrow purple circles. First and second femora with midventral black line extending full length of the joint and accompanied on each side by a black line in anterior third. First, second and third patellæ and tibiæ with a middorsal black line, first and second also with a prolateral and a retrolateral black line. Ends of first and second tibiæ black. Second and fourth metatarsi with a middorsal black line. First tarsi black, fourth dusky. Anterior coxæ yellow, posterior dark. Sternum dark. Abdomen white above with numerous black bars of various length (Figure 28). Venter yellow with spinnerets somewhat darker. Habitat: Wilcox camp on the San Lorenzo River.

Cyclosa V-notata sp. nov. (Figures 29-31).

Female. Total with cheliceræ 3.5875 mm. Carapace 1.295 mm. long, 0.703 mm. wide, with a typical wide procurved depression separating head from thorax. AME alone diurnal, situated on a tubercle. Anterior row of eyes recurved, posterior row straight. Ratio of eyes AME:ALE:PME:PLE = 2.5:2:2:2. AME separated from each other by 3/2.5 of their diameter, from ALE by their radius, from PME by their diameter. PME separated from each other by 3/2 of their diameter, from PLE by 1.5/2 of their diameter. Laterals subcontiguous, separated by a narrow line. Quadrangle narrower behind than in front in ratio 6.5:7, as wide behind as long. Clypeus convex, as high as quadrangle. *Cheliceræ* without boss. Margins little oblique and short. Promargin with 3 teeth, median tooth smallest. Retromargin with a single tooth. Fang small and stout. *Lip* slightly longer than wide in ratio 7:6, rounded and rebordered in front, not movable, but fused with sternum, although a procurved separation line is traceable. Lip reaches to middle of maxillæ. *Maxillæ* parallel,

long. Inner angle sharp, outer slightly produced. Scopula occupies entire truncature and reaches over upper edge. Serrula long. Outer margin of maxillæ distinctly concave. *Sternum* triangular,

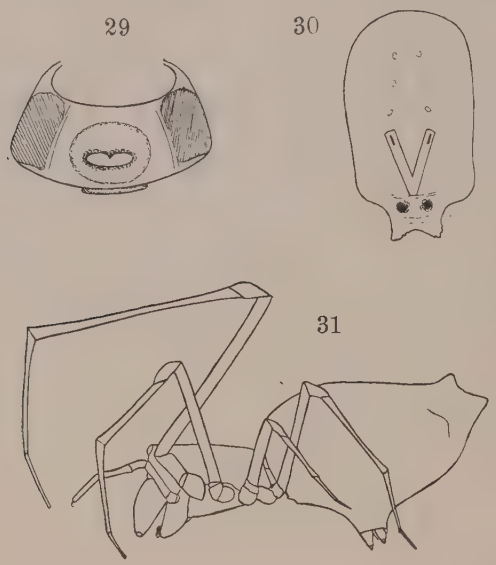


FIGURE 29, **Cyclosa V-notata** sp. nov., epigynum and lungs. FIGURE 30, Same, dorsal view of abdomen. FIGURE 31, Same, side view of female.

longer than wide in ratio 18 : 15, truncated at posterior end between the hind coxæ. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.255	2.501	1.271	0.697	6.724
II	1.148	1.271	0.738	0.492	3.649
III	0.615	0.615	0.410	0.328	1.968
IV	1.025	0.820	0.533	0.369	2.747

First femora curved so that they meet with their centers above the head whence they continue almost in contact with each other. Second and fourth femora also bent in the same sense, but not as strongly. Legs clothed with two kinds of hair forming alternating rows. The one kind is spinelike and is inclined to the axis of the leg at an angle of 20°. The second type is finer and forms an angle of 70°. Trichobothria difficult to see. Apparently a

single one on metatarsi beyond middle and another on tibiæ at base. Tarsi with a typical "drum." Upper claws with a single tooth, third claw smooth. Palpal claw smooth. *Abdomen* without shoulder tubercles, but with two tubercles near posterior end beyond which it becomes considerably constricted, ending in two small pointed tubercles with a pair of still smaller tubercles in front of these, so that in all there are three pairs of posterior tubercles of which one pair is lateral, one dorsal and one posterior. Spinnerets typical for the genus. Colulus well developed. Epigynum in a field of scleritised chitin, flat, transversely oval, with an opening almost three and a half times as wide as long (Figure 29). Color in alcohol: Carapace olive-brown with minute black dots and lines. Legs red-brown with yellow spot or bands. Cheliceræ, lip, maxillæ and sternum yellowish-brown. Abdomen above dark with a distinct and very sharply defined V-mark in front of dorsal tubercles. Anterior end lighter and iridescent with yellow. Sides iridescent with yellow and copper red. Venter iridescent with copper red. Surface between posterior tubercles and spinnerets dark with a few iridescent spots. Habitat: Wilcox camp on San Lorenzo River.

Acacesia cornigera sp. nov. (Figures 32-41).

a) Female. Total with cheliceræ 7.200 mm. Carapace 2.542 mm. long, 2.009 mm. wide, considerably narrowed in front, highest in the region of the thoracic groove which is longitudinal and deep. Posterior declivity steep. Anteriorly carapace slopes gently toward the eyes, then suddenly forms an eyetubercle on which the four median eyes are situated. In front of PME the carapace drops down almost vertically, so that AME are situated directly below PME (Figures 32 and 34). The laterals being sessile, the tubercle is rather narrow and the face, when viewed from in front, appears to be triangular. There are two rows of long, white, iridescent hairs between the median eyes, six hairs in each row. They are directed forward and outward. Clypeus concave, the eyetubercle projecting beyond it. Carapace clothed with fine, recumbent hair. Eyes in two rows. Viewed from in front anterior row is slightly curved downward, posterior row very strongly curved downward. Ratio of eyes AME:ALE: PME:PLE = 4.5:2.5:4:2.5, AME separated from each other by their diameter, from ALE by 8/4.5 of their diameter and from

PME by 2/4.5 of their diameter. PME separated from each other by 4.5/4 of their diameter and their axes form an angle of 70° between them. PME are separated from PLE by three diameters. Laterals are separated from each other by 2/2.5 of their diameter. Quadrangle very little wider in front than behind in ratio 11 : 10.5 and very little shorter than wide in ratio 10 : 10.5. *Cheliceræ* with a well-developed boss. Basal joint 1.11 mm. long,

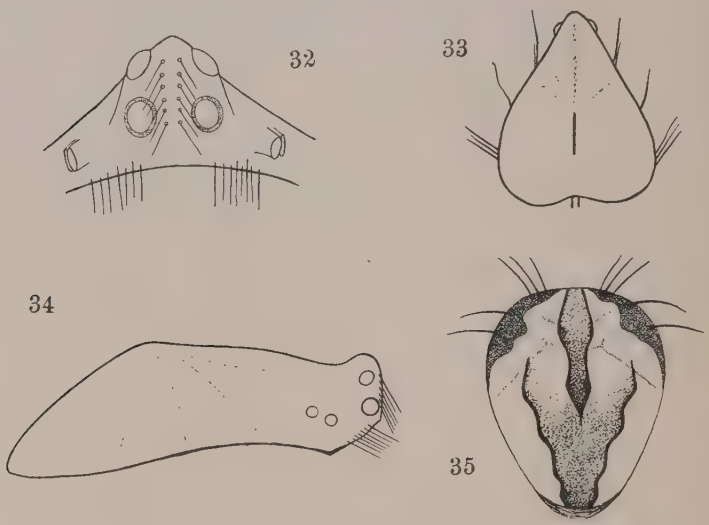


FIGURE 32, **Acacesia cornigera** sp. nov., front view of carapace of female. FIGURE 33, Same, dorsal view of carapace of male. FIGURE 34, Same, side view of carapace of female. FIGURE 35, Same, dorsal view of abdomen of male.

gradually attenuated toward end. Margins oblique. Promargin with six teeth of which the second proximal is by far the largest, then the first, third, fifth and sixth, while the fourth is the smallest and somewhat squeezed in as it were between its two neighbors. Second and third teeth are narrowly, but distinctly separated, first and second teeth are separated by a space equal to the base of the former. Retromargin with three fairly equal and equidistant teeth. No marginal scopulæ, but ventral surface as well as dorsal surface of cheliceræ sparsely clothed with erect bristles of black color. *Lip* as wide as long, strongly rebordered and almost pentagonal with a few black hairs at tip. *Maxillæ* parallel to

each other, wider at end than at base, with scopula along entire inner truncature and extending over anterior margin. Serrula long, reaching outer angle which is rounded. *Sternum* longer

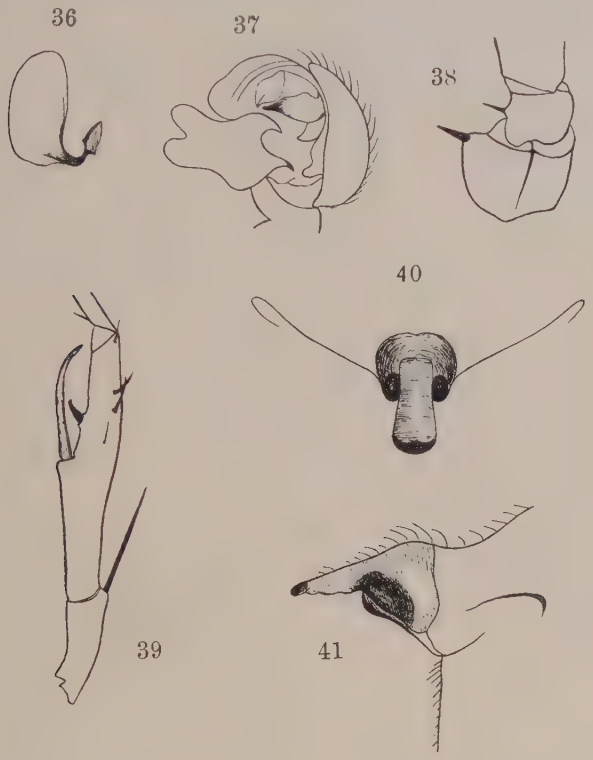


FIGURE 36, **Acacesia cornigera** sp. nov., cymbium of male palp, with apophysis. FIGURE 37, Same, right palp. FIGURE 38, Same, fourth right coxa and trochanter of male, showing spurs. FIGURE 39, Same, second right tibia and patella of male. FIGURE 40, Same, epigynum of female, front view. FIGURE 41, Same, epigynum of female, side view.

than wide in ratio 30:27, strongly emarginate in front, pointed behind, with rather deep excavations for second and third coxæ. Sternum clothed with long, erect hair. Trochanters all slightly emarginate. Second tibiæ slightly, but distinctly curved. Legs clothed with short, erect hair and short, spinelike hair. There is no special arrangement of the hair except for a prolateral and retrolateral row of spinelike hair on all metatarsi and tarsi, and

particularly on the first and second. Trichobothria only on tibiæ. Upper claws with five long teeth. Third claw smooth. Several spurious claws present. Palpal claw with a row of teeth increasing in length distally. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	4.320	5.088	3.456	1.056	13.920
II	3.840	4.512	3.072	0.960	12.384
III	2.208	1.728	1.248	0.576	5.760
IV	3.556	3.072	2.016	0.672	9.316

Spines. First leg. Femur—prolateral 1-1-1, elsewhere 0. Patella—dorsal 0-1 very small spine, prolateral 0, retrolateral 1, ventral 0. Tibia—dorsal 1r-1r, prolateral 0-1-1, retrolateral 0-1-2, ventral 1-1-1-1-1. Metatarsus—dorsal 0, prolateral a long row of spinelike hairs, retrolateral 0, ventral 1-2-1-1. *Second leg* same as first except that there is a row of 11 or 12 fine spines in mid-ventral line on femur. *Third leg.* Femur—dorsal 1 small spine a little before middle, prolateral 0, retrolateral 0, ventral a row of eight spines. Patella—dorsal 1-1, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-0, prolateral 0, retrolateral 1 apical, ventral 1-1. Metatarsus—dorsal 1, prolateral 0, retrolateral 0, ventral 2-1-1-1. *Fourth leg* same as third except: Femur 0. Tibia—prolateral 1 apical, ventral 1-1-1. Metatarsus—dorsal 0, ventral 1-0. Spines on *palp* long, arranged as follows: Femur—dorsal 1 apical. Patella—dorsal 1-1, prolateral 1. Tibia—dorsal 1, prolateral 1-1-0-1-1, retrolateral 1 apical. Terminal joint—dorsal 1, prolateral 2-2-1, retrolateral 1-1-1, ventral 1-1-1. Terminal joint longer than patella + tibia. *Abdomen* with a small median tubercle in front and two shoulder tubercles, and with a posterior median hump above the spinnerets. Epigynum quite prominent. When erected, it forms almost a normal to the surface of the venter. Scapus flat. Spinnerets typical for the family, not circumvallated. Color generally yellowish-brown. Abdomen below with a median longitudinal black band extending from genital groove to spinnerets. Dorsum greyish-buff with two dark spots in front of shoulder tubercles and a median folium of the same kind as in *Acacesia foliata.* Abdomen clothed with simple short and a few spinelike short hairs.

b) *Male.* Considerably smaller, more spiny and more vividly colored. Total 3.84 mm. Carapace 2.05 mm. long, 1.640 mm.

wide, of about the same shape as in female, but still narrower in front (Figure 33). Thoracic groove deep, longitudinal, with a depression gradually leading into it. Between the median eyes are only six bristles, but these as all others are much heavier and longer than in the female. There are two bristles on each side back of the lateral eyes, one bristle on edge of carapace between first and second leg, and three bristles on edge of carapace between second and third leg. The disposition of the eyes is somewhat different from that in the female as is the ratio AME:ALE: PME:PLE = 5.0:2.5:3.5:2.5. AME are separated from each other by 4/5 of their diameter, from ALE by 3/5 of their diameter and from PME by less than radius. PME are separated from each other by 4.5/3.5 of their diameter, from PLE by 8/3.5 of their diameter. Laterals are separated by less than their radius. Quadrangle wider in front than behind in ratio 12:9.5, as long as wide behind. Clypeus equal to diameter of AME. AME directly below PME. *Chelicerœ* weaker than in the female. Margins not well defined, teeth difficult to see, presumably same as in female, but to prevent injury to specimen teeth were not exposed. *Maxillœ* with a strong tooth at outer angle, which seems to be in some way functionally correlated with the chitinized lip of the proximal end of the femur of the palp. *Lip* as in female. *Sternum* narrower in front than in the female, produced into sharp points between each adjoining pair of coxæ. First coxa with a strong apical hook at retroangle, directed downward and slightly forward. Fourth coxa with a dark, strong, long spur. Fourth trochanter with a finer spur below (Figure 38). Second tibia modified (Figure 39), with a heavy spine in middle below, as long as half tibia, and a heavy short spine between this spine and the end of the joint. Both spines are curved. The pair of apical lateral tibial spines of the first leg have a kink near base. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	3.239	3.403	2.542	0.861	10.045
II	2.255	2.460	1.968	0.820	7.503
III	1.476	1.271	0.738	0.410	3.895
IV	2.255	1.927	1.435	0.615	6.232

Spines. First leg. Femur—dorsal 1-1-0-0-1, prolateral 1-1-1, retrolateral 0-0-1, ventral 0. Patella—dorsal 0-1, prolateral 0, retrolateral 1, ventral 0. Tibia—dorsal 0, prolateral 1-1-1-1, retro-

lateral 1-1-0-1, ventral 0-2. Metatarsus—dorsal 1-1, elsewhere 0. The row of prolateral hairs on first and second metatarsus and tarsus, as described in the female is wanting in the male. *Second leg* same as first, except: Femur—dorsal 1-1-1-1-1, prolateral 0-1, retrolateral 0, ventral a row of seven spines. Patella—dorsal 0-1, a very heavy spine, as long as patella itself. Tibia—dorsal 1r-0, prolateral 1-1-1-1, the first in middle, the following three heavy, short spines, ventral 1-0-1-1, first small, at base, second the immense curved spine, in middle, third the small curved spine, very dark. Metatarsus sinuous. *Third leg.* Femur—dorsal 1-1-1, prolateral 0-1, retrolateral 0-1, ventral variable number there being 3 midventral spines on the right leg and 7 on the left leg. Patella—dorsal 1-1, prolateral 0, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 0-1, retrolateral 0-1, ventral 1-1. Metatarsus—dorsal 1-0, prolateral 0, retrolateral 0, ventral 2-1-1-1. *Fourth leg* same as third, except: Femur—dorsal 1-1-1-1-1-1, ventral 1r-1r-1r-2-2-2. Patella—dorsal 0-1, prolateral 1. Tibia—dorsal 1-0, prolateral 1-1, retrolateral 1-1, ventral 1-1-1. Metatarsus—dorsal 1-1-1, prolateral 1-1, ventral 0-1. *Palp.* One very long middorsal spine on patella, elsewhere 0. Cymbium produced posteriorly into a narrow apophysis directed forward and outward (Figure 36). The structure of the bulb is shown in Figure 37. *Abdomen* (Figure 35) pointed behind, where it is considerably higher than the spinnerets, rounded in front without humps. Four long bristles are situated on each side on the black field of that portion of the dorsum which overlaps the carapace. On anterior edge of abdomen, where the latter forms the bend overlapping the carapace there is a transverse recurved row of four bristles and behind this another row of six bristles which is twice as long as the former, but less recurved. Color in alcohol: Generally olive-green and much darker than female. Eyetubercle orange-brown. Face lemon-yellow. Abdomen dark below with two longitudinal white marks, above buff with dark brown shoulder spots and the characteristic olive folium. First and fourth femora much darker than the others, except at base. Second and third femora are also darker in distal two thirds, but not as dark as first and fourth. There is a pair of longitudinal, parallel, narrow, light lines in distal third of first and second femora on their ventral surface. The fact that the folium is present in both sexes and in immature specimens on the abdomen, shows that this is an old

character independent of sex or age and that in conjunction with other characters may have even generic value. Habitat: Wilcox camp on San Lorenzo River.

Cyrtognata globosa sp. nov. (Figure 42).

Female. Total with chelicerae 4.8 mm. Carapace 1.640 mm. long, 1.270 mm. wide. Abdomen 2.460 mm. long, 2.132 mm. wide. Chelicerae (basal joint) 1.025 mm. long, 0.492 mm. wide above base. *Carapace* emarginate behind, narrowed in front to

FIGURE 42, **Cyrtognatha globosa** sp. nov., right chelicera of female from below.

the width of the eyegroup. Thoracic depression large, longitudinal, perfectly rectangular. Head elevated. Anterior row of eyes recurved, posterior row almost straight (very slightly recurved). Eyes equal. Posteriors equidistant. AME closer together than they are from ALE. Laterals contiguous. Posterior eyes separated by a little more than their diameter. Quadrangle wider than long in ratio 11:9, wider behind than in front. Clypeus equal to one and one half diameters of AME. *Chelicerae* strongly divergent, forming a right angle between them, suddenly constricted at base and with a heavy chitinous ridge in place of a boss. Inner apical angle with a strong tooth. Margins very long. Promargin with eight teeth increasing in size distally, penultimate tooth displaced toward retromargin. Retromargin also with eight

teeth, the two distal teeth very stout. Fang long and distinctly bent from middle on (Figure 42). *Lip* wider than long in ratio 8:6, truncated and rebordered at end, does not reach middle of maxillæ. *Maxillæ* parallel. Truncature almost parallel with outer edge and therefore almost at right angles with the distal margin. Scopula dark grey, very dense and even. Entire distal margin is occupied by the serrula which is elevated on a narrow ridge extending slightly beyond outer margin and forming a sharp though short projection. *Sternum* more or less triangular, truncated between fourth coxæ which are separated by their diameter. Between the first and second, and between the second and third coxæ the angles of the sternum are produced into narrow chitinous strips extending to a scleritized band of epimerites situated between the carapace and the coxæ, somewhat as in *Dysdera*. Sternum very little longer than wide in ratio 23:21. Fourth femora much more slender than the others and curved laterally. First metatarsi curved dorsiventrally downward. True spines wanting, but all hair spinelike in appearance and arranged in regular rows on all tibiæ, metatarsi and tarsi. Each hair forms an angle of 30° with the axis of the leg. At greater intervals between these hairs much shorter erect hairs form an angle of 85° to 80° with the axis of the leg. Trichobothria only on tibiæ, situated in two rows. Upper claws with a series of about 12 teeth. Third claw strongly bent, smooth. Palpal claw smooth. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.8875	3.5000	3.0625	1.3125	10.7625
II	2.6250	3.0625	2.6250	1.1375	9.4500
III	1.7500	1.5750	1.4875	0.7000	5.5125
IV	2.4500	2.1875	2.0125	0.8750	7.5250
Palp	0.8750	0.8750	0.7875		2.5375

Abdomen evenly rounded in front and behind, clothed with short black hair and scattered, long, black bristles. Distance between lung openings and genital slit a little greater than distance between the latter and the spinnerets. Between tracheal spiracle, which lies immediately in front of the spinnerets, and the genital slit, but closer to the latter than to the former, there is a narrow transverse sclerite. Genital slit slightly procurved. Colulus well developed. Spinnerets terminal, typical of the family. Color in alcohol: Carapace yellowish-brown with a median dark triangle

occupying the entire length of the head and extending to the end of thoracic depression. Anteriorly the triangle is as wide as the eyearea. Lateral declivities of the head yellow. Thoracic part with a pair of converging dark bands. Legs and palpi yellowish-brown, cheliceræ more reddish and darker. Abdomen above with four irregular longitudinal dark bands in anterior half, ending in an irregular transverse dark band behind which there is a complicated though not clearly defined folium extending to spinnerets and inclosing pearl-white dots of the same type as found also between the central pair of the anterior dark bands. Coxæ yellow. Sternum, lip, maxillæ olive-green. Venter with a broad, longitudinal dark band extending from anterior end to spinnerets and bordered on each side by a narrow, light band. Habitat: Wilcox camp on San Lorenzo River.

Mimicosa gen. nov.

Carapace flat, wide. Head slightly elevated. Sides of head slanting from PLE, but almost vertical in front. Eyes of first row strongly curved downward, much smaller than eyes of second and third row. Quadrangle of medians much narrower in front than behind and wider than long. First row of eyes much wider than second, but much narrower than third. AME farther from ALE than from each other. Cheliceræ parallel, both margins with three teeth. Lip slightly wider than long. Sternum much wider than long and anterior coxæ wide apart. Legs 1243, long, slender, devoid of scopulæ, but with numerous and very long spines. First tibiæ with four pairs of spines below, first metatarsi with five pairs of spines below. All tibiæ with a dorsal row of curved "cilia" near base. Two dorsal spines on third and fourth tibiæ. Type **M. spinosa.**

Mimicosa spinosa sp. nov. (Figures 43-47).

Male. Total 5.568 mm. Carapace 2.460 mm. long, 2.050 mm. wide between second and third coxæ, greatly narrowed in front where it measures only 0.962 mm. Thoracic groove longitudinal, very long and deep, beginning considerably behind head the limit of which is plainly shown by the cephalothoracic sulci and because it is slightly elevated above the otherwise flat carapace. Arrangement of eyes still more exaggerated than in *Azilia,* approaching that of some Pisauridæ. Viewed from in front first row is

strongly bent downward, second row bent upward. Width of first row, measured in a straight line, 0.555 mm., that of second row 0.740 mm. Ratio of eyes AME : ALE : PME : PLE = 2.25 : 2 : 3.75 : 3.75. AME separated from each other by a little more than their diameter, from ALE by two diameters of the latter, from PME by a little less than the diameter of the PME.

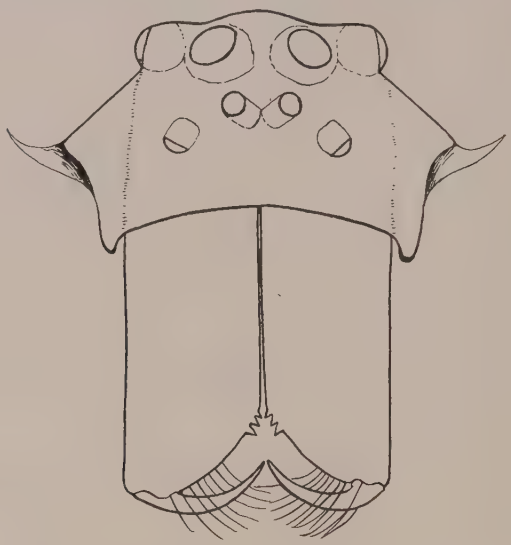


FIGURE 43, **Mimicosa spinosa** sp. nov., front view of face and cheliceræ.

PME separated from each other by a little more than their diameter, from PLE by one and one half of their diameter. Laterals separated from each other by 5 diameters of the ALE. Quadrangle wider than long in ratio 11 : 9, much wider behind than in front in ratio 11 : 7. ALE directed downward and slightly outward, PLE backward and slightly upward. Clypeus in front of AME almost as high as quadrangle in ratio 8 : 9, in front of ALE only 5/9 of the length of the quadrangle. Sides of face slanting from PLE to the level of ALE where they meet with the lateral edge, but almost vertical in front (Figure 43). *Cheliceræ* (Figures 43 and 45) parallel, rather slender. Basal joint 1.036 mm. long, 0.407 mm. wide in middle. Boss well developed. Margins

strongly oblique, each with 3 sharp teeth. On both margins, near articulation of fang long, curved, stiff and rather thick hairs. Fang thin and strongly curved, with a distinctly serrated proedge. *Lip* wider in middle than long in ratio 10:9, with almost parallel sides and squarely truncated end. Lateral excavations at base so deep that the width of the lip at base is only 7/9 of its length.

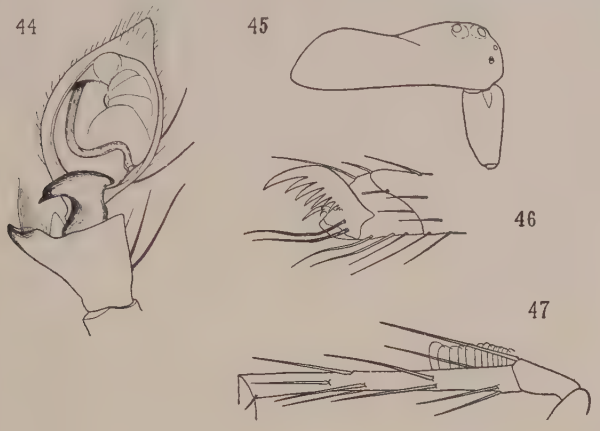


FIGURE 44, **Mimicosa spinosa** sp. nov., right palp. FIGURE 45, Same, carapace in side view. FIGURE 46, Same, end of first left tarsus. FIGURE 47, Same, fourth tibia with a row of curved trichobothria or "cilia."

Lip articulated to maxillæ a little before middle and does not reach middle of maxillæ. *Maxillæ* parallel, a little more than twice as long as wide. Outer edge slightly concave. Truncature short, oblique. Scopula with a tuft of long hair followed by a regular brush of short hair. Serrula short, just reaching to outer angle which is rounded. *Sternum* wider than long in ratio 32:26, widely truncated in front, so that anterior coxæ are very far apart, while posterior coxæ are contiguous. All trochanters deeply notched. Hair on legs and body simple and sparse. Spines, except lateral ones on femora, very long, often overlapping and with the end of one spine reaching to the base of the second following spine. At the base of all tibiæ there is a dorsal row of from 11 to 13 trichobothria which are curved backward (Figure 47) like the so-called "cilia" on the third tibiæ of *Mangora* or on the fourth femora of *Leucauge* and *Mecynometa*.

These cilia are accompanied on each side by 2 or 3 similar cilia or trichobothria. On the metatarsi the trichobothria form a single row, but on the tarsi they form two rows and are also curved backward. The legs are very slender. First metatarsi slightly curved. Claws three, situated on an onychium (Figure 46). Upper claws curved, with 6 teeth which may be preceded by 2 or 3 very small teeth at base. Third claw less curved, with a single tooth. Several heavy bristles on the onychium, resembling spurious claws, but somewhat differently structured inasmuch as they are not serrated in the usual manner, but minutely spinose all over. *Legs* 1243.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	3.362	0.984	3.772	4.182	1.230	13.530
II	3.362	0.984	3.526	4.100	1.148	13.120
III	2.870	0.861	2.706	3.280	0.943	10.660
IV	3.280	0.861	3.075	3.895	1.066	12.177
Palp	1.480	0.555	0.555	1.925		4.515

Spines. First leg. Femur—dorsal 1-1-1, the first two very long, the third quite short; prolateral 1-1-1-1-1, the first two quite short, the others long; retrolateral 1-1-1-1 short spines; ventral 11 pairs of bristles. Patella—dorsal 1 near end, very long, elsewhere 0. Tibia—dorsal 1-1 long spines, the first quite near base, the second 3/5 from base; prolateral 1 near middle, retrolateral 1 near middle, ventral 2-2-2-2, last pair very short. (Dorsal "cilia" described above.) Metatarsus—dorsal 2 before middle; prolateral 1-1, first near base, second before middle; retrolateral 1-1, same way; ventral 2-2-2-2-2, last pair quite small. *Second leg* same as first except: Femur—prolateral 1-1-1-1 all short, retrolateral 1-1-1; Tibia—ventral 2-2-2-2-2-2. Metatarsus—dorsal 0. *Third leg.* Femur—dorsal 1-1-1, third pair short; prolateral 1-1-1-1 short spines, retrolateral 1-1, ventral 9 pairs of bristles. Patella—dorsal 1 very long spine at end, elsewhere 0. Tibia—dorsal 11-1, the first near base, the second beyond middle; prolateral 1-1, retrolateral 1-1; ventral 2-2-2-2, last pair short; Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-2, last pair short. (Dorsal cilia on tibiæ described above.) *Fourth leg* same as third, except: Femur—retrolateral 0-1. *Palp.* Femur—dorsal 1-1, first beyond middle and long, second at end, short; prolateral 1-1, retrolateral 1, ventral 0. Patella—dorsal 1 bristle near base,

prolateral 1 bristle, elsewhere 0. Tibia—prolateral 2, elsewhere 0. Terminal joint—prolateral 2-1, elsewhere 0. Tibia of pedipalp with a retrolateral and a ventral apophysis. The former has the shape of a hollow half cone and has a tooth at its base near the dorsal surface of the tibia. The ventral apophysis has the shape of a pickax with short handle. Terminal joint is quite narrow at its base, widening suddenly. Embolus in the shape of a short, fine, black needle arising close to middle of bulb at right angles to its surface. *Abdomen* narrow and long, more than twice as long as high in anterior third, in a general way resembling the abdomen of *Tetragnatha laboriosa*. Spinnerets terminal, cylindrical. Anterior pair contiguous, short and stout, posterior pair but slightly longer. Preservation of abdomen rather poor, integuments strongly wrinkled. In the type specimen white shows in numerous spots below the skin. In the other specimens abdomen is almost entirely white above, the median heart-mark excepting. Rest of spider yellow. This species is in many ways related to *Azilia* and probably belongs in the same group. At the same time not one of its structures is characteristic of the Family Argiopidæ, while the lip, the trochanters, and especially the eyes are altogether more of the Lycosid than of the Argiopid type. Habitat: Trijoles, Canal Zone.

Family Mimetidæ

Gelanor heraldicus sp. nov. (Figure 48).

Female. Total without cheliceræ 3.825 mm. Carapace 1.445 mm. long, 1.445 mm. wide, very high, especially at the juncture of

Figure 48, **Gelanor heraldicus** sp. nov., epigynum.

head with thorax in front of thoracic groove which is transversely oval. From this point carapace slopes forward and backward.

AME large, their axes form between them an angle of 90°. AME separated from each other by their radius, from ALE by more than their diameter. Laterals contiguous, equal, also equal to PME. PME are separated from each other by more than their radius, from PLE by two of their diameters. Ratio of eyes AME:ALE:PME:PLE = 5:3:3:3. Anterior row of eyes recurved, posterior row procurved. Quadrangle much wider in front than behind in ratio 11:7, as long as wide in front and therefore much longer than wide behind in ratio 11:7. Clypeus less than diameter of AME. *Chelicerœ* long, narrowed at base, without boss. Promargin with a row of seven slender teeth, retromargin with a single tooth which has the shape of a tubercle. Scopulæ wanting, but a few hairs are on outside surface of chelicerœ, near promargin. *Lip* slightly wider than long in ratio 8:7, reaching middle of maxillæ. *Maxillæ* long and sub-parallel. *Sternum* triangular, produced between fourth coxæ, much longer than wide. *Legs* 1243.

Spines. First leg. Femur—dorsal 1-1-3, elsewhere 0. Tibia —dorsal seven or eight long spines separated in the manner typical for the family by shorter ones forming intermediate rows of from 3 to 4 in number, elsewhere 0. Metatarsus with 4 long spines separated by small ones increasing in number distally and followed beyond the fourth long spine by a row of small spines diminishing in size to the end of the joint. *Second leg* same as first, except that there are only two long spines on metatarsus. *Third leg* only with isolated spines: 1 on patella and 1 tibia near base (both dorsal), also a pair of dorsal spines on metatarsus near base. *Fourth leg* same as third except that the metatarsal spines are wanting. Third and fourth leg are much smaller and more slender than first and second. Palpi very slender and spinose. Palpal claw smooth. A row of trichobothria on all tibiæ, metatarsi and tarsi. Abdomen wider than long, widest in region of spinnerets. Colulus present. Epigynum with four receptacles forming a trapeze (Figure 48). Color in alcohol: Carapace yellow with dusky separation lines between head and thorax, forming a letter V which does not reach the edge of the carapace. A pair of dusky lines run from groove toward base of anterior legs, widening toward the periphery. A dusky triangular area back of the groove. Eyes, except AME, in a field of carmine color. Abdomen above with a transverse white band margined by dark reddish-

black and followed by three narrow, transverse, wavy lines of carmine. Sternum yellow in anterior half, dark grey in posterior half, the demarkation line between the two fields very sharp and situated on the level between second and third coxæ. Cheliceræ, lip and maxillæ dark yellow. Coxæ light yellow. Abdomen with a white band on each side, forked anteriorly, narrowed posteriorly where the two bands reach the posterior angles. Field between these bands red. Third and fourth legs light yellow. First and second leg yellow with carmine and black spots on femora and a black median line on tibiæ below. Distal ends of first and second tibiæ black. Habitat: Wilcox camp on San Lorenzo River.

Family Thomisidæ

Synaema fuscomandibulatum sp. nov. (Figure 49).

Male. Total 2.720 mm. Carapace 1.275 mm. long, 1.275 mm. wide. All eyes on separate tubercles, but the tubercles of ALE and PLE contiguous. PME smallest, next AME, next PLE. ALE largest, their diameter twice that of PME. AME separated

Figure 49, **Synæma fuscomandibulatum** sp. nov., right palp of male.

from each other by two diameters and from ALE by the same distance. PME nearer each other than PLE in ratio 9:8 and nearer ALE than PLE in ratio 5.5:7. Quadrangle wider than long in ratio 9:8, narrower in front than behind. Clypeus 6/8

of the length of the quadrangle. Arrangement of bristles on carapace and abdomen very regular. Especially may be mentioned four bristles on the carapace, one in center of each crimson spot. Legs very spinose. Palp shown in Figure 44. Tibia of palp with two apophyses. Color very characteristic: Carapace dark yellow with iridescent white eyetubercles, broad crimson marginal band and four small crimson spots in middle. Cheliceræ very dark. Abdomen lighter than carapace, with two irregular, converging crimson bands in anterior half, followed by a right and left transverse band, the two barely separated from each other in middle, then follow two very short transverse bands confluent laterally with a narrow procurved transverse band and finally a still narrower transverse line. Sternum and palpi light yellow. Lip and maxillæ darker. Abdomen below dark yellow, with six dark crimson parallel lines on sides, directed obliquely inward toward the spinnerets. Coxæ dark yellow. First trochanters crimson. First femora very dark yellow with crimson ventral surface. Same coloration on the first patellæ. First tibiæ dark yellow in proximal half, crimson in distal half. Same coloration on metatarsi, except that they are lighter in color. First tarsi yellow. Second tibia, metatarsus and tarsus are colored as in first leg. Second trochanter and femur are yellow, patella yellow with a crimson spot. Third and fourth leg yellow with small crimson spots on patella, at base and end of tibia and at end of metatarsus. The species is closely allied to, but distinct from *S. cirripes* Cambridge. Habitat: Cerro Flores.

Family Selenopidæ

No less than sixteen species of the Genus *Selenops* have been described from various parts of North and South America, and I have in my collection a seventeenth species, *S. jamaicensis* sp. nov., from Jamaica, which I shall describe in another paper dealing with the spider fauna of that island. It is unfortunate that for six of the seventeen species the order of legs is not given, for this seems to be an important and constant group character. The following list will help the grouping of species. It will be observed that in four species the order of legs for the male is the same as for the female, while in *S. nigromaculatus* Keyserling the two sexes show a marked difference. Whether this is a

natural difference, or whether the two sexes do not belong to the same species cannot be decided without new material. Hitherto in five species the order of legs was known only in the female sex.

Species	Sex	Order of legs
S. bifurcatus Banks	male and female	not mentioned
S. debilis Banks	female	not mentioned
S. galapagoensis Banks	female	not mentioned
S. marginalis F. Cambridge	male and female	not mentioned
S. minutus F. Cambridge	male and female	not mentioned
S. morosus Banks	female	not mentioned
S. aissus Walckenaer	female	2314
S. argentinus Tullgren	female	2314
S. insularis Keyserling	female	2314
S. jamaicensis sp. nov.	female	2314
S. cocheleti Simon	male and female	2341
S. spixii Perty	male and female	2341
S. mexicanus Keyserling	male and female	2341
S. ecuadorensis Berland	female and young male	2413
S. nigromaculatus Keyserling	female	2413
S. nigromaculatus Keyserling	male	3241
S. celer MacLeay	female	3214
S. confusus nom. nov. Syn. *S. aissus* Keyserling	female	2134

The systematic position of the three species, *S. aissus* Walckenaer, *S. celer* MacLeay and *S. confusus* nom. nov. may be understood from a study of the original descriptions. Walckenaer in describing the specimen to which he gave the name *S. aissus* states explicitly on page 547: "Pattes: la seconde paire la plus longue, la troisième ensuite, la quatrième est la plus courte." MacLeay, referring to the differences between the subgenus *Aissus* and his own sub-genus *Hypoplatea,* makes the following remark on page 6, Vol. 2, Ann. Nat. Hist. 1838, "The difference between the West Indian subgenera Aissus and Hypoplatea is that in the former the first pair of feet are the longest, whereas in Hypoplatea it is the penultimate pair"... This statement is difficult to reconcile with the original description given by Walckenaer. It is possible that MacLeay had a new species before him which he identified as *S. aissus* and which has never been rediscovered since. The specimen which Keyserling described as *S. aissus* and for which

I propose here the new name *S. confusus* is undoubtedly different from Walckenaer's *S. aissus*. An error of statement is out of the question because of the careful measurements given by Keyserling.

Selenops aissus Walckenaer, Ins. Apt., 1837, Vol. 1, p. 547.
nec *Selenops aissus* Keyserling, 1883.
? nec *Selenops aissus* auctorum.
Figures 50-54.

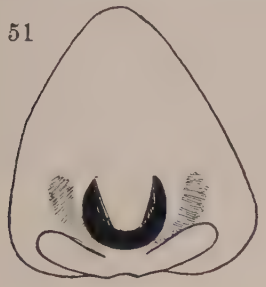
I have in my collection four mature females of about the same size, all collected by me in Jamaica. One of these specimens was caught on a Pandanus plant in the Castleton Botanical Gardens in

FIGURE 50, *Selenops aissus* Walckenaer, eyegroup of female from Jamaica.

March, 1905, the other three under the bark of Pimento trees in Malvern in the last days of April of the same year. One of these specimens has been selected for description, the others fully agreeing with it. I have further one mature female from Remedios, Panama, and one from Sona, Panama, captured by Mr. Valentine in March, 1924. Both agree fully with the specimens from Jamaica. Since the description of the Jamaican specimen was already finished in manuscript before the Panama specimens came into my possession, I give here its description. The slight variation in the structure of the epigynum is best dealt with by referring to Figures 51-54.

Female. Total with cheliceræ 17.0 mm. Carapace 6.8 mm. long, 7.2 mm. wide, considerably narrowed in front, so that head is only 3.8 mm. wide. Eyegroup occupies entire width of head (Figure 50). First row composed of six eyes which are almost equidistant and form a slightly recurved line. Ratio of eyes AME : ALE : PME : PLE = 9 : 9.5 : 7 : 15. PME oval; in the ratio given here, their long axis has been used in comparison with

the diameter of the other eyes. Since the figure has been made with the aid of the camera lucida, the disposition of the eyes may be easily understood without detailed description. Sternum longer



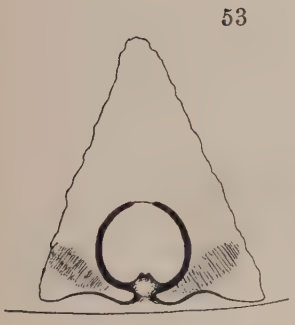


FIGURE 51, *Selenops aissus* Walckenaer, epigynum of a female from Remedios. FIGURE 52, Same, epigynum of a female from Sona. FIGURE 53, Same, epigynum of a female from Jamaica. FIGURE 54, Same, epigynum of another female from Jamaica.

than wide in ratio 3.8 : 3.4. It is slightly emarginate or notched at posterior end. Promargin of cheliceræ with three teeth, retromargin with two teeth. Legs 2314.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	6.4	8.5	5.2	2.2	22.3
II	7.5	9.9	5.5	2.4	25.3
III	7.6	8.7	5.4	2.2	23.9
IV	6.9	7.7	5.0	2.0	21.6

Coxæ of fourth pair considerably longer than the others.

Spines. First leg. Femur—dorsal 1-1-1 long spines, and 1r-1r-1r short spines of which the first is situated 2/5 from base; prolateral 1-1, retrolateral 0, ventral 0. Patella 0. Tibia—ventral 2-2-2, elsewhere 0. Metatarsus—ventral 2-2, elsewhere 0. *Second leg* same as first. *Third leg* same as first, except: Tibia—ventral 2-2. *Fourth leg* same as third. Scopulæ heaviest on legs of first pair where they extend even to the end of the tibia as far as base of distal spines. On hind legs the scopulæ are poorly developed. Claws concealed by ungual tufts. Terminal joint of palp with about a dozen long, curved spines. Palpal claw with 11 teeth. Abdomen 9.4 mm. long, 5.2 mm. wide anteriorly, 7.0 mm. wide in its widest place, *i. e.,* in the region of the last two dots. Thoracic groove longitudinal. Sulci well developed. Integuments with hair of various kinds: long, brown hair; shorter and much stouter hair especially on cheliceræ, carapace and dorsum of abdomen; stout, white hair with blunt end forming a fringe along anterior edge of abdomen. Epigynum in a more or less triangular field. Color in alcohol: Carapace and legs reddish-brown, legs with faint indications of darker markings on femora and tibiæ, which are more distinct in other specimens. Carapace with marginal black line. Dorsum of abdomen greyish-yellow with almost black margin. Venter lighter. Lip and maxillæ with light end. Cheliceræ slightly darker than carapace. Eyes surrounded by black. Entire spider very flat. Egg-cocoon large, flat, elliptical, 45 x 25 mm. consisting of a flat rim by which it is attached to the wood and a central receptacle containing numerous eggs. Habitat: West Indies (Martinique and Jamaica) and Panama. (Other localities not certain, because of the confusion of this species with *confusus.*)

Family Sparassidæ

Defectrix, gen. nov.

Carapace as in *Sparianthis.* Both rows of eyes recurved, anteriors smaller than posteriors, equidistant and equal in size. PME equal to PLE and nearer to them than to each other. Quadrangle wider behind than in front, slightly wider than long. Clypeus equal to radius of AME. Promargin of cheliceræ with 4 or 5 teeth, retromargin with 2 teeth. Lip twice as wide as long,

not reaching middle of maxillæ. Sternum transversely elliptical, wider than long. Legs 4123. All tarsi, metatarsi and tibiæ scopulate below and on sides. Anterior tibiæ with two pairs of ventral spines; anterior metatarsi with a single pair of ventral spines at base. Type **D. defectrix.**

Defectrix defectrix sp. nov. (Figures 55-61).

Immature female. Total with cheliceræ 4.420 mm. Carapace 1.785 mm. long, 1.700 mm. wide, considerably narrowed in front where the clypeus is only 0.510 mm. wide. In the region of

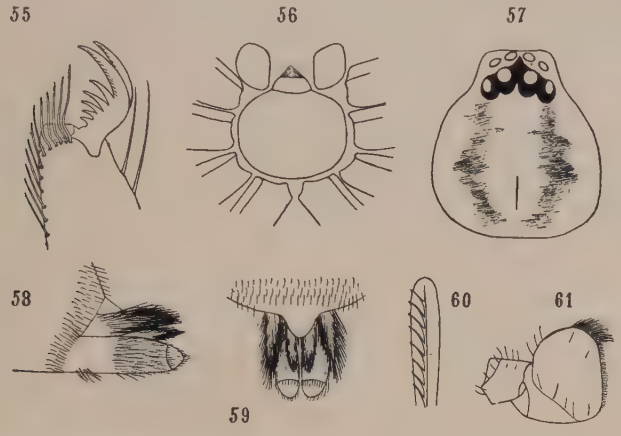


FIGURE 55, **Defectrix defectrix** sp. nov., claws of second leg. FIGURE 56, Same, sternum, lip, maxillæ and coxæ. FIGURE 57, Same, carapace, dorsal view. FIGURE 58, Same, side view of spinnerets. FIGURE 59, Same, spinnerets from above. FIGURE 60, Same, end of a hair from a tarsal scopula highly magnified. FIGURE 61, Same, right maxilla from below, with trochanter of palp.

posterior row of eyes the carapace is 0.765 mm. wide, while the eyerow measures 0.630 mm. Generally speaking, the carapace is rather low, being highest in the middle of the thoracic groove which is longitudinal and linelike. From this point the carapace slopes gently forward and steeply backward. Its sides are clothed with hair of two kinds: short stiff and long fine hair. The middle of carapace is bare. Eyes in two rows of four each, both recurved. Anterior row shorter than posterior. Each eye of anterior row surrounded by a narrow black ring, eyes of posterior row in a common black field which is festooned posteriorly (Figure 57).

Ratio of eyes AME : ALE : PME : PLE = 4 : 4 : 5.5 : 5.5. AME separated from each other by 1.5/4 of their diameter, from ALE by the same distance and from PME by 2.5/4 of their diameter. Laterals separated by 3/4 of the diameter of ALE. PME separated from each other by 2/5.5 of their diameter, from PLE by 1/5.5 of their diameter and from ALE by their radius. Quadrangle wider behind than in front in ratio 12 : 9, wider than long in ratio 12 : 11. Clypeus equal to the radius of AME. ALE nocturnal, all others diurnal. All eyes round. Cheliceræ rather small, cone-shaped, with a weak yellow boss. Margins oblique, promargin with four or five teeth, retromargin with two teeth. Scopula only on promargin. *Lip* much wider than long in ratio 12 : 6, trapeze-shaped. Its anterior end with a fringe of converging bristles. Lip does not reach middle of maxillæ. *Maxillæ* parallel to each other, short and wide (Figure 61). Inner edge straight, fringed with hair which almost has the appearance of a scopula. Truncature short with a poorly developed true scopula. Outer angled rounded. Serrula black, not long. Trochanter so inserted that its upper edge, in side view, is beyond middle of maxilla while its lower edge is one-fifth from base. *Sternum* wider than long in ratio 34 : 27, transversely elliptical (Figure 56), slightly emarginate anteriorly for the reception of the lip. First pair of coxæ almost as far apart as second and somewhat farther apart than third. Fourth pair of coxæ contiguous, sparsely clothed with stiff, moderately long hair. All trochanters notched. Ends of metatarsi with trilobate membrane. Claws 2, strongly curved, with five teeth (Figure 55). Palpal claw more slender, but of the same type. True claw tufts wanting, but the end of the tarsal scopula forms pseudo-ungual tufts. Legs clothed with stiff hair. Heavy scopulæ below and on sides of all tarsi and metatarsi, extending in the shape of four longitudinal bands almost to base of tibia. These scopulæ are formed by peculiar hair which is arranged in regular rows and is iridescent on account of numerous notches along the edge of their flattened end (Figure 60). *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	1.870	2.635	1.275	0.890	6.610
II	1.870	2.465	1.275	0.890	6.500
III	1.785	2.230	1.275	0.890	6.180
IV	2.040	2.720	2.125	0.890	7.775
Palp	0.690	0.900	0.900		2.490

Tibia of palp twice as long as wide and but little longer than patella.

Spines. First leg. Femur—dorsal 1-1-1, the distal spine small; prolateral 0-1-1, elsewhere 0. Patella—dorsal 1-1 very fine, short bristles, elsewhere 0. Tibia—ventral 2-2-0, elsewhere 0. Metatarsus—ventral 2 near base, elsewhere 0. *Second leg* same as first. *Third leg* same as first, except: Femur—prolateral 1-1-1, retrolateral 1-1-1. Tibia—prolateral 1-1, ventral 1-2-0. *Fourth leg* same as first, except: Femur—retrolateral 1 at end. Tibia—dorsal 0-1, prolateral 1-1, retrolateral 1-1, ventral 1-2-0. Metatarsus—prolateral 1-1, ventral 2-0-1. *Palp.* Femur—dorsal 1-1, prolateral 0-1, elsewhere 0. Patella—dorsal 1-1, prolateral 1, elsewhere 0. Tibia—dorsal 2-2, prolateral 1-1, retrolateral 0-1, ventral 0. Terminal joint—dorsal 1 near base, prolateral 1-1, retrolateral 1-1, ventral 0-2-3. Trichobothria numerous on all legs, situated in four rows on tibiæ and metatarsi, in two rows on tarsi. Colulus absent, but its place marked by a few hairs. Spinnerets six on an elevation, anteriors slightly longer and much heavier than posteriors, contiguous, with short, conical second joint. Medians slender, cylindrical. Posteriors apparently with but a single joint and are separated from each other by the width of the anal tubercle (Figures 58 and 59). Tracheal spiracle immediately in front of spinnerets. Abdomen clothed with simple hair of two kinds: stiff, long hair and fine, long, recumbent hair. General color in alcohol pale yellow, with two dusky longitudinal, irregular bands on carapace and a median dusky spot on dorsum of abdomen in front. All femora with two dusky, indistinct rings. Habitat: Panama City.

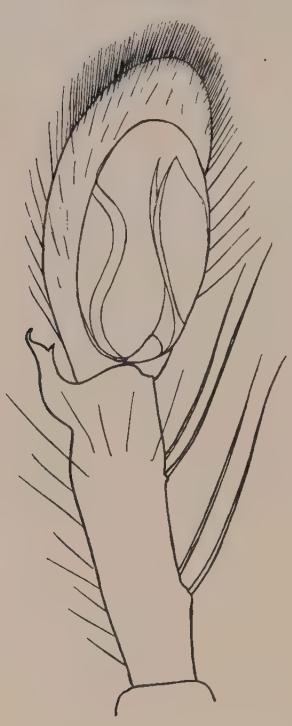
The specimen is somewhat pressed out of shape by having been packed too closely with other spiders, making it impossible to decide whether the legs are prograde or laterigrade. It is difficult to place this spider in any known genus. It has some characters in common with the Sparianthidæ. The fact that it has been captured in Panama City makes it possible that it is an importation from the East.

Family Clubionidæ

Clubiona sigillata sp. nov. (Figures 62-64).

a) Male. Total with cheliceræ 5.355 mm. Carapace 2.125 mm. long, 1.530 mm. wide, oval. Eyes occupy almost full width of

carapace which measures in the eyeregion 0.935 mm. Thoracic groove longitudinal, short. Eyes in two rows. Anterior row recurved, posterior row slightly recurved and longer than anterior. AME alone diurnal. All eyes round. Ratio of eyes



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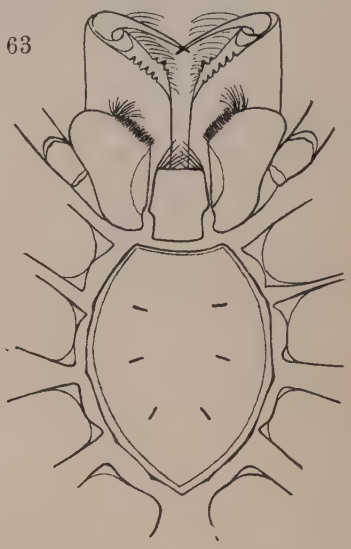

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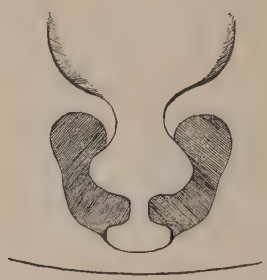

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FIGURE 62, **Clubiona sigillata** sp. nov., right palp from below. FIGURE 63, Same, sternum, lip, maxillæ and cheliceræ of male. FIGURE 64, Same, epigynum of female.

AME : ALE : PME : PLE = 4 : 4.5 : 4 : 4.5. AME separated from each other by their radius, from ALE by half of their radius, from PME by 3/4 of their diameter. PME separated from each other by their diameter, and from PLE by the same distance. Laterals separated by 1.5/4.5 of their diameter. Quadrangle

wider behind than in front in ratio 14:11 and wider than long in the same ratio. Clypeus equal to 1.5/4 of the diameter of AME. 1 bristle behind each PLE and a fringe of six bristles on edge of clypeus. *Cheliceræ* with very weak, yellow boss. Basal joint 0.890 mm. long. Margins oblique. Promargin with 5 teeth of which the distal two are barely visible, while of the proximal three the median is by far the largest. Retromargin with 5 teeth, the first tooth considerably smaller than the rest. Scopula only on promargin. *Lip* longer than wide in ratio 12:9, reaching slightly beyond middle of maxillæ. Lateral excavations one third the total length of the lip. Sides almost parallel and anterior truncature straight. *Maxillæ* long, with both sides concave. Outer angle rounded. Oblique depression wanting. Scopula with a long tuft followed by shorter hair which occupies the entire surface of the truncature. Serrula very long, curved, reaching to outer edge of maxillæ. *Sternum* oval, longer than wide in ratio 39:28, narrowed in front, anterior truncature procurved. Posteriorly sternum is pointed, hind coxæ almost contiguous. Lateral margins of sternum distinctly rebordered and on the sternum itself there are three pairs of quite distinct linear sigilla, each pair opposite an intercoxal space and considerably removed from edge of sternum (Figure 63). (A similar case is presented by *Oonops reticulatus*.) Third and fourth trochanters notched. Tarsal scopulæ wanting, but claw tufts well developed. Trichobothria numerous, in two rows on all tibiæ, metatarsi and tarsi. *Legs* 4213.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	1.881	2.640	1.716	0.792	7.029
II	2.442	3.102	1.749	0.792	8.085
III	1.716	2.079	1.716	0.528	6.039
IV	2.310	2.904	2.739	0.660	8.613

Spines. First leg. Femur—dorsal 1-1-1, prolateral 0-1-1, retrolateral 0-1-0, ventral 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 1 fine bristle at base, prolateral 1-1, retrolateral 0, ventral 2-2-2, the apical pair small. Metatarsus—dorsal 0, prolateral 0, retrolateral 1 at base, ventral 2 at base. *Second leg* same as first, except: Femur—retrolateral 0-1-1. Tibia—dorsal 1-0-1, retrolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 0-1-1, retrolateral 0-1-1, ventral 0. Patella

—dorsal 1-1 fine bristles, retrolateral 1, elsewhere 0. Tibia—dorsal 1-0-1, the distal a bristle, prolateral 1-1, retrolateral 1-1, ventral 1-1-2. Metatarsus—dorsal 0-1-2, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2. *Fourth leg* same as third, except: Tibia—ventral 2-2-2. *Palp.* Femur—dorsal 0-1-2, prolateral 1 at end, elsewhere 0. Patella—dorsal 1-1 bristles, prolateral 1 bristle, elsewhere 0. Tibia—prolateral 2-2, elsewhere 0. Tibia three times as long as wide. Tibial apophysis with two small sharp hooks. *Tarsal claws* 2, with 5 strong teeth. *Abdomen* twice as long as wide. Tracheal spiracle immediately in front of spinnerets. Colulus wanting. Spinnerets 6, anteriors contiguous, cylindrical, about as long as posteriors. Terminal joint of posterior spinnerets short, coneshaped. Anal tubercle very long, pointed, reaching to middle of posterior spinnerets. Anterior end of abdomen clothed above with long, stout hair, rest of body and legs with long, stiff hair and shorter, fine recumbent hair. All hairs simple. Color in alcohol generally light yellow with cheliceræ, lip and maxillæ much darker. Three rows of indistinct grey spots on the back of the abdomen, extending all way to spinnerets. The species is closely related to *C. tricuspis* Cambridge. Habitat: Wilcox camp on San Lorenzo River.

b) Female. Total with cheliceræ 6.375 mm. Carapace 2.110 mm. long, 1.650 mm. wide. *Legs* 4231.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	1.445	1.785	0.935	0.500	4.665
II	1.615	2.125	1.060	0.510	5.310
III	1.360	1.700	1.275	0.500	4.835
IV	1.870	2.295	2.040	0.520	6.725

Spines. First leg same as in male, except: Femur—retrolateral 0. Tibia—dorsal 1-0-1 fine bristles, prolateral 0, ventral 2-2-0. Metatarsus—retrolateral 0. *Second leg* same as first, except: Femur—prolateral 1 at end. *Third leg* same as in male, except: Femur—prolateral 1 at end, retrolateral 1 at end. *Fourth leg* same as third. Cheliceræ as in male but the two distal teeth on promargin more distinct and the distal tooth on retromargin smaller. Other characters and coloration as in male. Sigilla plainly visible. Palp without claw, but terminal joint with dorsal 2-0, prolateral 1-1, retrolateral 1-1 and ventral 0-2 spines. Epigynum of a peculiar shape shown in Figure 64.

Cragus mandibulatus sp. nov. (Figures 65-67).

a) Male. Total with cheliceræ 3.40 mm. Carapace 1.320 mm. long, 1.000 mm. wide. Basal joint of cheliceræ 0.760 mm. long. Abdomen 1.360 mm. long, 0.723 mm. wide. Thoracic groove wanting. Carapace rather high, projecting posteriorly so that posterior declivity is saddle-shaped with a fringe of stiff hair at highest edge, otherwise glabrous, truncated anteriorly where it is

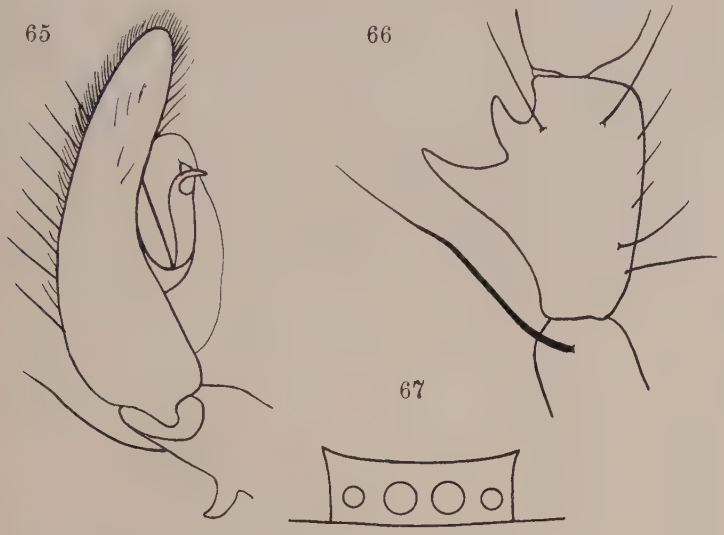


FIGURE 65, **Cragus mandibulatus** sp. nov., right palp from outside. FIGURE 66, Same, tibia of left palp of male. FIGURE 67, Same, epigynum of female.

only 0.510 mm. wide. Eyes in two rows, anterior row recurved, posterior row procurved and longer than anterior in ratio 15:12. AME alone diurnal. Ratio of eyes AME:ALE:PME:PLE = 1.75:3:3:3. AME separated from each other by 1.5/1.75 of their diameter, from ALE by 1/1.75 of their diameter and from PME by 4/1.75 of their diameter. PME separated from each other by two diameters and from PLE by one diameter. Laterals separated by radius. Quadrangle wider behind than in front in ratio 8.5:5, wider than long in ratio 8.5:7. Clypeus 1/1.75 of the diameter of AME. *Chelicerœ* long and slender, diverging, with a few long hairs. Margins very oblique. Boss wanting. Promargin with three equal and equidistant teeth, spaced rather

wide apart, first being at proximal end of margin and last in middle. Retromargin with five small teeth, the last of which is somewhat in advance of the others. Scopula only on promargin, scanty, but of long hair. Fang somewhat sinuous at base. *Lip* longer than wide in ratio 9:6, reaching beyond middle of maxillæ, its end strongly emarginate. Lateral excavations at base present. *Maxillæ* quite long and more than twice as long as wide. Outer edge concave. Truncature with scopula almost at right angles with upper edge, which, too, though rounded is almost at right angles with outer edge. Scopula scant. Serrula reaching outer edge. Maxillæ well visible also from above, being together wider than cheliceræ. Palp is inserted near base. *Sternum* much longer than wide in ratio 28:18, narrowed in front to the width of the lip, posteriorly produced between hind coxæ which are separate. The ends of the produced portion are confluent with the edge of the carapace. Legs very slender, with rather long spines. Scopulæ wanting, but claw tufts well developed. *Legs* 1423.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	1.870	3.045	2.890	1.105	8.910
II	1.275	1.785	1.360	0.510	4.930
III	0.935	1.190	1.020	0.423	3.568
IV	1.530	1.955	1.615	0.510	5.610

Spines. First leg. Femur—dorsal 1-1-1, elsewhere 0. Patella—dorsal 1-1, elsewhere 0. Tibia—dorsal 1-0-1, prolateral 1-1, retrolateral 1-1, ventral 1p-1r-1p-1r. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-0. *Second leg* same as first, except: Tibia—ventral 1-2. *Third leg* same as first, except: Tibia—dorsal 1-0-1, prolateral 0-1, retrolateral 0-1, ventral 0. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-0-1, ventral 2-0-2. *Fourth leg.* Femur—dorsal 1-1-1, prolateral 0-0-1, retrolateral 0-0-1, ventral 0. Patella—dorsal 1-1, elsewhere 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 1-1. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1-1, ventral 1-1-0. Claws 2, with a row of 12 teeth. Claw tufts composed of 12 scales. Also two pairs of serrated bristles on tarsi. Trichobothria in a single row on all tibiæ, metatarsi and tarsi. Legs clothed with simple hair. *Abdomen* long, with very long, cone-shaped anal tubercle at the end of which are hairs. Spinnerets 6, posteriors much longer and more slender than anteriors, their

second joint quite distinct, but much shorter than first. Anteriors contiguous. Tracheal spiracle somewhat nearer genital fold than spinnerets. *Palp* with long curved femur. Tibia only two and a half times as long as wide. Tibial apophysis large, situated in middle of tibia, with a smaller apophysis between the larger one and the apex of the joint. Spines as follows: Femur—dorsal 1-1, prolateral 0-1, elsewhere 0. Patella—dorsal 1-1 bristles, the second very long, elsewhere 0. Tibia—dorsal 0-1, prolateral 2-1, retrolateral 0-1, elsewhere 0. Color in alcohol: Generally light yellow with sternum and coxæ almost white. Black spots back of each eye. Abdomen below with a grey longitudinal median band extending from petiolus almost to spinnerets.

b) Female. Total with cheliceræ 2.80 mm. Carapace 1.254 mm. long, 0.924 mm. wide. Cheliceræ slightly converging, attenuated at end, basal joint 0.460 mm. long. *Legs* 1423.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	1.615	2.635	2.125	0.980	7.355
II	1.105	1.615	1.020	0.380	4.120
III	0.810	0.980	0.765	0.340	2.895
IV	1.360	1.700	1.445	0.470	4.975

Spines considerably longer than in male. *First leg.* Femur—dorsal 1-1-1, prolateral 1 at end, elsewhere 0. Patella—dorsal 1-1, elsewhere 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2. *Second leg* same as first except: Femur—dorsal 1-1-1, otherwise 0. *Third leg.* Femur—dorsal 1-1-1, otherwise 0. Patella—dorsal 1-1, otherwise 0. Tibia—dorsal 1-0-1, prolateral 0, retrolateral 0-1, ventral 1-1. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 1 at base and a cluster of small apical spines. *Fourth leg.* Femur—dorsal 1-1-1, prolateral 1 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1, elsewhere 0. Tibia—dorsal 1-0-1, prolateral 1-1, retrolateral 1-1, ventral 1-1-0. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 1-0-1. *Palp.* Femur—dorsal 1-1, prolateral 1 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1, the second very long, prolateral 1, elsewhere 0. Tibia—dorsal 1-0, prolateral 2-1, retrolateral 0-1. No spines on terminal joint. Femur equal in length to tibia + patella and equal to terminal joint. Palpal claw minute, smooth. Epigynum in the shape of a

transverse rectangular small plate with four round receptacles forming a transverse row, central pair larger than the lateral ones. Color generally paler than in male. Median dark band on venter of abdomen wanting. Habitat: Wilcox camp on San Lorenzo River.

Sillus longispina F. Cambridge (Figures 68-69).

Male. Total with cheliceræ 3.910 mm. Carapace 1.615 mm. long, 1.360 mm. wide, truncated anteriorly where it is only 0.720 mm. wide; rounded posteriorly. Thoracic groove short,

FIGURE 68, *Sillus longispina* F. Cambridge, right palp from outside. FIGURE 69, Same, right palp from below.

longitudinal. Posterior declivity begins behind thoracic groove and is rather steep. Carapace clothed with fine, dark, recumbent hair. A few bristles on the eyearea, apparently without definite arrangement. Eyearea smaller than width of carapace in that region in ratio 19:26. All eyes round, but only AME diurnal. Anterior row of eyes slightly recurved, posterior row procurved and longer than anterior. Ratio of eyes AME: ALE: PME: PLE

= 2 : 3.5 : 3.25 : 3.25. AME separated from each other by their radius, from ALE by the same distance and from PME by two diameters. PME are separated from each other by 3/3.5 of their diameter, from PLE by 2.5/3.5 of their diameter. Laterals are separated by 1/3.25 of their diameter. *Chelicerœ* with weak yellow boss. Basal joint 0.720 mm. long. Margins oblique. Promargin with 4 teeth, retromargin with 5 teeth. Scopula only on promargin. *Lip* longer than wide in ratio 11 : 8, reaching beyond middle of maxillæ, excavate laterally at base, slightly emarginate at end. *Maxillæ* parallel to each other, outer edge concave. They are more than twice as long as wide, dilated and rounded anteriorly. Scopula occupies entire surface of truncature. Serrula long, extending to outer edge. *Sternum* heart-shaped, slightly longer than wide in ratio 30 : 27, distinctly emarginate in front, pointed behind between hind coxæ which are separated by a little less than their diameter. All trochanters notched. Tarsi without scopulæ, but claw tufts well developed. Trichobothria in two rows on all tibiæ, metatarsi and tarsi. Claws two with 4 or 5 teeth. Claw tufts composed of 6 pairs of scales. *Legs* 1423.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.125	3.145	1.955	0.850	8.075
II	2.090	2.805	1.785	0.850	7.530
III	1.445	1.615	1.530	0.595	5.185
IV	2.090	2.550	2.380	0.765	7.785

Tibia of palp almost three times as long as wide, patella shorter than tibia in ratio 10 : 14.

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1-1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 bristles, elsewhere 0. Tibia—dorsal 1-1 bristles, prolateral 1-1-1, retrolateral 1-1, ventral 2-2. Metatarsus—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2. *Second leg* same as first. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1, ventral 0. Patella—dorsal 1-1 bristles, prolateral 0, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2, distal pair small. Metatarsus—dorsal 0, prolateral 1-1-2, retrolateral 1-1-2, ventral 2-2-2. *Fourth leg* same as third, except: Metatarsus—dorsal 1. *Palp.* Femur—dorsal 1-2, prolateral 1 distal, elsewhere 0. Patella—dorsal 1-1 bristles, prolateral 1 bristle, elsewhere 0. Tibia—prolateral 1-1 bristles, retrolateral 1 bristle,

elsewhere 0. Embolus long needle-like, arising on inside about one-third from end of bulb. A hooked conductor is opposite to the embolus and directed toward it. Tibial apophysis half as wide at end as at base, constriction quite sudden, giving the apophysis the aspect of an organ consisting of two parts. Tracheal spiracle in middle of abdomen. Colulus wanting. Anterior spinnerets contiguous, shorter and stouter than posterior ones. Anal tubercle long, in the shape of an isosceles triangle. Color pale yellow, with cheliceræ, lip and maxillæ slightly darker. Body and legs clothed with simple hair. A few curved, stout hairs on dorsal surface of abdomen in front. Habitat: Costa Rica, Guatemala, Panama. Specimen here described from the Wilcox camp on San Lorenzo River.

Syrisca pulchra sp. nov. (Figures 70-73).

Female. Total with cheliceræ 10.0 mm. Carapace 4.165 mm. long, 2.975 mm. wide in widest place, 2.125 mm. wide behind eyes. Thoracic groove longitudinal, deep. Eyegroup small, occupies a little more than one-third of the width of carapace in the eye-region. Eyes in two rows all diurnal, round. Anterior row slightly recurved, posterior very slightly procurved—slightly longer than anterior row. Ratio of eyes AME : ALE : PME : PLE = 5.5 : 6 : 5.5 : 6. AME separated from each other by 2/5.5 of their diameter, from ALE by the same distance and from PME by double that distance or 4/5.5 of their diameter. PME separated from each other by 4/5.5 of their diameter, from PLE by the diameter of the latter. Quadrangle as long as wide, wider behind than in front in ratio 15 : 13. Laterals separated from each other by 2.5/6 of their diameter. Clypeus 3/5.5 of the diameter of AME. Along the edge of the clypeus a row of black bristles. Carapace clothed with two kinds of hair: short, black, recumbent and slightly longer, erect, black hair. Lateral margins of carapace with a fringe of much longer, light-colored hair, directed forward. *Chelicerœ* powerful, with boss, geniculated and rather slender toward end, clothed with black bristles. Promargin with three weak teeth of which median is largest, retromargin with 2 very small teeth separated from each other by a large space. *Lip* longer than wide in ratio 22 : 20, sides excavated in basal third, truncated anteriorly almost straight, this truncature being only 12/20 of the width of the lip. End of lip with a few black bristles.

Lip reaches just to middle of maxillæ. *Maxillæ* with very slight, oblique impression. Outer angle rounded. Truncature oblique. Scopula brown, composed of a tuft of long hair followed by

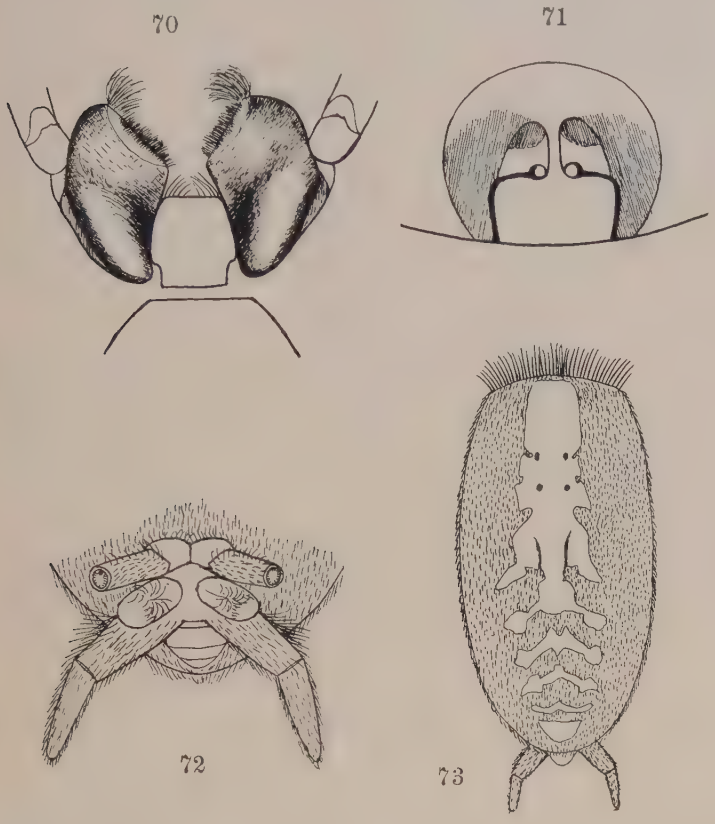


FIGURE 70, **Syrisca pulchra** sp. nov., maxillæ and lip. FIGURE 71, Same, epigynum. FIGURE 72, Same, spinnerets from below. FIGURE 73, Same, dorsal view of abdomen.

shorter hair which does not form a narrow line. Palpi inserted near middle. Serrula long. *Sternum* oval longer than wide in ratio 25 : 20, truncated in front, pointed behind, not rebordered, fairly flat, sparsely clothed with black hair. Anterior truncature is less than half the width of the sternum (ratio 9 : 20). Posterior coxæ nearly contiguous. First and second pair of trochanters

slightly notched, third and fourth pair strongly notched. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.720	4.165	1.785	1.275	9.945
II	2.720	3.740	1.700	1.275	9.435
III	2.550	3.230	1.785	1.275	8.840
IV	3.315	4.420	3.315	1.445	12.495

First and second tarsi and metatarsi scopulate to base. Scopulæ entire. Third tarsus scopulate to base, third metatarsus scopulate to base only on prolateral surface, while on retrolateral surface it is scopulate in distal third. Fourth tarsus lightly scopulate to base, scopula divided longitudinally by a narrow line of setæ. Hair on legs long. Trichobothria in two rows on tibiæ, metatarsi and tarsi. Claws two, with 5 teeth. Palpal claw with 5 teeth.

Spines on legs and palpi heavy. *First leg.* Femur—dorsal 1-1-0, prolateral 1 distal, elsewhere 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 1-0-1 fine bristles, elsewhere 0. Metatarsus 0. *Second leg* same as first, except: Femur—prolateral 0-1-1, both close together. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 1-0-1 fine bristles, prolateral 1-1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0-2-2, prolateral 1-1-0, retrolateral 1-1-0, ventral 2-0-2. *Fourth leg* same as third except: Femur—retrolateral 0-0-1, Tibia—dorsal 1-1, but the first is a true spine, while the second is a bristle, retrolateral 1-1-1. Metatarsus—dorsal 2-2-2, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2. *Palp.* Femur—dorsal 1-1-2, elsewhere 0. Patella—dorsal 1-1, prolateral 1, elsewhere 0. Tibia—dorsal 1-1, prolateral 2-1, elsewhere 0. Terminal joint—dorsal 1, prolateral 2-1, retrolateral 1, ventral 2 small spines near apex. *Abdomen* long, rather hairy. Tracheal spiracle near spinnerets. Colulus wanting. Spinnerets six, posterior ones two-jointed, long; second joint pointed, cylindrical, as long as first joint. Anterior spinnerets very little separated at base (Figure 72). Epigynum shown in Figure 71. Color in alcohol: Carapace and cheliceræ reddish-brown. Legs yellowish-brown. Abdomen dark greyish-brown with a median yellow herring-bone mark extending full length of the back. Anterior edge of abdomen with a fringe of long hair. Venter greyish-yellow, sternum, coxæ, maxillæ and lip reddish-brown. Habitat: Remedios.

Syrisca hirsuta sp. nov. (Figures 74-76).

a) Male. Total with cheliceræ 7.14 mm. Carapace 3.400 mm. long, 2.550 mm. wide in widest place, 1.360 mm. wide just behind eyegroup. Abdomen 3.400 mm. long, 1.700 mm. wide, cylindrical. Eyegroup much narrower than width of carapace in that region. Eyes in two rows, anterior row slightly recurved, posterior row very slightly procurved and longer than anterior

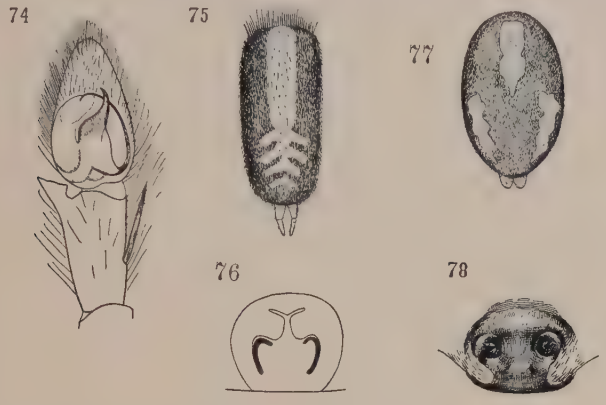


FIGURE 74, **Syrisca hirsuta** sp. nov., right palp from below. FIGURE 75, Same, dorsal view of abdomen of male. FIGURE 76, Same, epigynum. FIGURE 77, **Corinna luteomaculata** sp. nov., dorsal view of abdomen of female. FIGURE 78, Same, epigynum.

one. Ratio of eyes AME : ALE : PME : PLE = 4.5 : 4.5 : 4.5 : 4.5. AME separated from each other by 1.5/4.5 of their diameter, from ALE by the same distance, from PME by twice that distance. PME separated from each other by 3/4.5 of their diameter, from PLE by the same distance. Laterals separated from each other by 1.5/4.5 of their diameter. Quadrangle wider than long in ratio 12 : 11, wider behind than in front in the ratio 12 : 10. Thoracic groove longitudinal, deep. A row of black bristles along the anterior edge of clypeus. A few scattered black bristles behind eyes and a middorsal row of black bristles beginning behind eyegroup and ending with a pair in front of thoracic groove. Carapace clothed with black, recumbent, short hair. Lateral margins with a fringe of light-colored, long hair directed forward. *Cheliceræ* clothed with long black bristles, in front. Promargin with 3 teeth of which median is largest.

Retromargin with 2 small widely separate teeth. Scopula on promargin only, yellowish-brown. *Lip* longer than wide in ratio 17 : 15, with basal lateral excavations, distinctly rebordered at tip. *Maxillæ* with very light oblique impression. Outer angle rounded. Scopula with a tuft of brown hair followed by irregularly arranged hair which however does not extend over the edge of the truncature. Serrula dark brown, long. Palp inserted near middle of maxillæ. *Sternum* oval, longer than wide in ratio 21 : 17, truncated in front, pointed behind, slightly but distinctly rebordered in front and on sides, sparsely clothed with long, erect, brown hair. Coxæ with same type of hair. Posterior coxæ contiguous. First and second trochanters slightly notched, third and fourth deeply notched. Legs very hairy and spines numerous and long. Tarsi and metatarsi of first and second leg and tarsus of third leg scopulate to base with entire scopulæ. Metatarsus of third leg scopulate only in distal half and scopula bisected longitudinally by a band of long setæ. Fourth tarsi scopulate to base, their scopula divided longitudinally by two rows of setæ. Trichobothria long, numerous, in two rows on all tibiæ, metatarsi and tarsi. Claw tufts well developed. Claws 2, with a row of 5 teeth.

Spines. First leg. Femur—dorsal 1-1, prolateral 0-1-1, both near each other and close to end of joint. Elsewhere 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 1, very long bristle near end, prolateral 1 near base, retrolateral 0, ventral 2-2-2. Metatarsus—ventral 2 near base, elsewhere 0. *Second leg* as first, except: Femur—prolateral 1-1-1, Tibia—prolateral 1 near end. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1, fine bristles, elsewhere 0. Tibia—dorsal 1-1-1, the third a fine bristle, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2. Metatarsus—dorsal 2-2, prolateral 1-1, retrolateral 1-1, ventral 2-2. *Fourth leg* same as first, except: Femur—retrolateral 0-1-1, Metatarsus—dorsal 2-2-2, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2, the two spines between the proximal and distal pair not forming a real pair, but the retrospine considerably in advance of the prospine. *Palp.* Femur—dorsal 1-1-2, prolateral 1 near end, retrolateral 1 near end, ventral 0. Patella—dorsal 1-1, prolateral 1, elsewhere 0. Tibia—dorsal 1, prolateral 2-1, elsewhere 0. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.550	3.655	1.870	1.530	9.605
II	2.295	3.315	1.700	1.095	8.405
III	2.040	2.975	1.700	1.095	7.810
IV	2.890	4.165	3.145	1.445	11.645

Tibial apophysis of palp and structure of bulb represented in Figure 74. Embolus originating at base of bulb. *Color* in alcohol: Carapace smoky red-brown. Legs yellowish-brown except anterior tibiæ, metatarsi and tarsi, which are dark brown, and corresponding segments of the second leg, which are darker than in the other legs, but not as dark as in the first leg. Abdomen black with a yellow median band which is straight, with parallel sides as far as middle of abdomen. From here on posteriorly the band assumes the shape of the "herring bone" with four lateral yellow ribs. A fringe of long, black hair along the anterior edge of abdomen, dorsum clothed with comparatively long, black hair. Spinnerets light yellow. Posteriors two-jointed, second joint as long as first. These spinnerets, as in the preceding species, are much longer than the anteriors which are sub-contiguous, being separated by less than their diameter. Tracheal spiracle near spinnerets. Color of venter: Lip, maxillæ and cheliceræ yellowish-brown. Sterum deep yellow. Legs yellow except anterior patellæ, tibiæ, metatarsi and tarsi which are dark smoky brown. Abdomen light yellow.

b) Female. Total with cheliceræ 6.3 mm. Carapace 2.380 mm. long, 2.000 mm. wide. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	1.615	2.125	1.020	0.850	5.610
II	1.615	2.125	1.020	0.850	5.610
III	1.530	1.870	1.020	0.850	5.270
IV	1.955	2.720	2.125	1.020	7.800

Spines. First leg. Femur—dorsal 1-1, prolateral 0-0-1, apical, elsewhere 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 1-1, second bristle very long, elsewhere 0. Metatarsus 0. *Second leg* same as first, except: Femur—prolateral 0. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1-1, retrolateral 0-0-1, ventral 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 1-0-1 fine bristles, prolateral 1-1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 2-2, prolateral 1-1-0, retrolateral 1-1-0, ventral 2-2.

Fourth leg, same as third, except: Femur—prolateral 1-1, retrolateral 0. Tibia—dorsal 1-1-1, the distal one a long, erect bristle. Metatarsus—dorsal 2-2-2, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2. In other respects female agrees with male and has the same hairy appearance, the same color and pattern on abdomen. Although the specimen is smaller than the male and comes from a different locality, there seems to be little doubt that they belong to the same species. The slight differences in the arrangement of spines may be easily accounted for by the difference of sex. Epigynum (Figure 76) much like that of *S. pulchra.* Habitat: Male from Cerro Iglesia, female from Panama City.

Corinna luteomaculata sp. nov. (Figures 77 and 78).

Female. Total with cheliceræ 11.9 mm. Carapace 3.485 mm. long, 2.890 mm. wide in the widest place, 1.870 mm. wide in the region of the eyes. Thoracic groove longitudinal, deep. Edge of carapace finely rebordered and fringed with evenly spaced, fine, short hair elevated at right angles to the margin. Anterior row of eyes slightly procurved, posterior row more procurved and a little longer. Laterals prominent. All eyes round, diurnal. Ratio of eyes AME : ALE : PME : PLE = 7 : 7 : 6 : 7. AME separated from each other by 5/7 of their diameter, from ALE by 4/7 of their diameter and from PME by 5/7 of their diameter. PME separated from each other by 7/6 of their diameter, from PLE by 10/6 of their diameter. Laterals separated by 2/7 of their diameter. Quadrangle as wide in front and behind as it is long. Clypeus equal to the diameter of AME. One middorsal bristle in front of AME and one on each side in front of ALE (*i. e.* three bristles all together), two bristles on anterior edge of clypeus in front of AME, two bristles posterior to and to the inside of AME, one bristle on each side between laterals, one bristle back of each of the four posterior eyes and 1-1 middorsal bristles posterior to the eyegroup. *Chelicerœ* long and slender, geniculated, with strong boss and long margins. Basal joint 1.615 mm. long, 0.850 mm. wide at base. Promargin with 3 teeth near proximal end of it, median strongest. Retromargin with 5 equal and equidistant teeth beginning on the level of the distal tooth of the promargin and extending to end of margin. Scopula on promargin only, composed of long heavy, light-colored hair.

On under surface of cheliceræ an oblique narrow band of dark hair extending from middle of basal joint inward and downward. *Lip* wider than long in ratio 21 : 18, slightly emarginate at tip, with lateral excavations in basal third, distinctly articulated to maxillæ. Lip does not reach middle of maxillæ. *Maxillæ* with parallel sides, neither concave on outside, nor with oblique impression on surface. Outer angle rounded. Scopula only on truncature, composed of a tuft of long brown hair and a narrow brush of short hair. Serrula very long, extending in a semicircle to the edge of the maxilla. *Sternum* distinctly rebordered, as long as wide, broadly truncated in front, pointed behind. Anterior truncature only 37/60 of maximum width of sternum. Sternum fairly convex, shiny, sparsely clothed with erect black hair. Anterior coxæ wide apart, hind coxæ contiguous. All trochanters notched. All tarsi with ungual tufts and a fine scopula reaching to base, barely noticeable on hind legs. There is a well visible scopula at end of third and fourth metatarsi. A very light scopula is also present on first and second metatarsi, reaching almost to base. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	3.315	4.420	2.720	1.445	11.900
II	3.145	3.995	2.465	1.275	10.880
III	2.975	3.400	2.465	1.190	10.030
IV	3.740	4.590	3.470	1.445	13.245

Spines. First leg. Femur—dorsal 1-1, prolateral 1, elsewhere 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—ventral 2-2-2-2, elsewhere 0. Metatarsus—ventral 2-2, elsewhere 0. *Second leg* same as first. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1, ventral 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-0, retrolateral 1-1-0, ventral 2-2-2. *Fourth leg* same as third, except: Femur—prolateral 0-1, retrolateral 0-1. Metatarsus—retrolateral 1-1-1. *Palp.* Femur—dorsal 1, apical, prolateral 1 apical, retrolateral 0, ventral a not quite regular row of 8 bristles. Patella—dorsal 1-1 fine bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1, prolateral 1-1-1, elsewhere 0. Terminal joint—prolateral 2 near base (one above the other), retrolateral 1, elsewhere 0. Palpal claw toothed. Claws on legs two, strongly curved. with 3 teeth,

concealed by claw tufts. Legs clothed with simple black and plumose light-colored hair. Trichobothria on all tibiæ and metatarsi in a single row, on all tarsi in two rows. Tracheal spiracle close to spinnerets. Colulus well developed. Spinnerets about equal in length, anteriors contiguous. Both anteriors and posteriors with very short terminal joint. Two large spinning tubes or spigots on each posterior spinneret, and three on each median, but none on anterior spinnerets. Abdomen clothed above with both simple and plumose hair, below only with simple hair. Epigynum in the shape of a large, red, transversely oval plate with two circular depressions. Color in alcohol: Carapace red-brown, mottled with black. Cheliceræ red-brown. Palpi and legs yellow. Abdomen brown with three yellow longitudinal stripes of which the median begins at anterior edge and ends in middle of abdomen, while the lateral stripes begin at middle and extend almost to spinnerets. Below cheliceræ, lip, maxillæ and sternum reddish-brown, legs yellow, abdomen greyish-brown with a pair of indistinct parallel longitudinal stripes. Spinnerets yellowish-brown. Habitat: Cerro Flores.

Corinna bulbosa F. Cambridge. Biol. Centr. Amer. Vol. 11. PI. V. ff. 3, 4.

Female. Total with cheliceræ 10.5 mm. Carapace 3.825 mm. long, 3.060 mm. wide, very little narrowed in front being 2.380 mm. wide in the region of the eyes. The carapace is convex, granular, without hair, with edge slightly rebordered and minutely serrated. Thoracic groove longitudinal, small. Head elevated. Cephalothoracic sulcus visible because of the elevation of the head, otherwise negligible. A procurved semilunar depression across the entire carapace and concentric with cephalothoracic sulcus is present 2/3 back of thoracic groove. Eyes in two rows, both rows very slightly procurved, first row shorter than second. Lateral eyes on slight elevation. Ratio of eyes AME:ALE: PME:PLE = 10:6:8:9. AME separated from each other by 7/10 of their diameter, from ALE by 6/10 of their diameter and from PME by their radius. Laterals separated by 5/6 of the diameter of ALE. PME separated from each other by 12/8 of their diameter, and by the same distance from PLE. Quadrangle wider than long in ratio 25:22, wider behind than in front in ratio 25:22. Clypeus distinctly rebordered, 9/10 of the diameter

of AME. ALE separated from edge of clypeus only by their diameter. The two sclerites in front of clypeus highly chitinized, granular, of the same appearance as carapace and cheliceræ. *Chelicerœ* powerful, with anterior surface granular. Margins long, oblique. Promargin with 3 strong teeth, median tooth largest. Retromargin with 4 strong, equal and equidistant teeth. Scopula on promargin only, composed of stiff, comparatively short, yellowish-brown hair. Underside of cheliceræ smooth. *Lip* very slightly wider than long in ratio 24: 23, truncated in front, with lateral excavations in basal third, smooth, with a few long bristles at end. Lip not quite reaching middle of maxillæ. *Maxillæ* slightly inclined over lip. Each maxilla with parallel outer and inner sides, very obliquely truncated at end, without oblique impression on surface. Scopula soft, brown, occupying a straight line on the white truncature. Serrula black, long, visible only from above. Surface of maxillæ smooth. *Sternum* ovoid, with anterior wider end squarely truncated. Sides strongly rebordered. Sternum convex, granular, hairless, much longer than wide in ratio 23: 19. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	3.230	4.165	2.805	1.700	11.900
II	2.975	3.825	2.550	1.445	10.795
III	2.550	3.060	2.550	1.190	9.350
IV	3.485	4.590	3.825	1.360	13.260

No scopula on tarsi, but claw tufts well developed. All tarsi distinctly more slender toward base. Claws concealed, with four teeth. Palpal claw also with 4 teeth. All tibiæ and metatarsi cylindrical. Trichobothria comparatively small and difficult to see, although legs are almost free from hair. Apparently they are in two rows on tarsi and possibly so on metatarsi and tibiæ.

Spines, except femoral, rather short. *First leg.* Femur—dorsal 1-1-1, prolateral 1 near end, elsewhere 0. Patella—dorsal 1-1 fine, short bristles, elsewhere 0. Tibia—dorsal 1 fine, short bristle near end, prolateral 0, retrolateral 0, ventral 1-2-2-2-2. Metatarsus—dorsal 1 fine, short bristle near base, prolateral 0, retrolateral 0, ventral 2-2. *Second leg* same as first, except: Femur—dorsal 1-1, prolateral 1-1, both beyond second dorsal. Tibia—retrolateral 1 fine, short bristle near middle, ventral 1-2-2. *Third leg* same as first, except: Tibia retrolateral 1-1, ventral 2-2-2.

Metatarsus retrolateral 1 near base, ventral 2-2-3. *Fourth leg* same as third. *Palp.* Femur—1-1, prolateral 1, elsewhere 0. Patella—dorsal 1-1 fine bristles, prolateral 1, elsewhere 0. Tibia—prolateral 1-1, elsewhere 0. Terminal joint—prolateral 1-1, elsewhere 0. Insertion of palp near base of maxilla. Tracheal spiracle near spinnerets. Colulus well developed. Spinnerets 6, anteriors sub-contiguous, about same length and barely stouter than posteriors. Petiolus well visible. Epigynum as figured by F. Cambridge, in the shape of a small, almost round, median depression. Anteriorly to this a pair of large, dark, oval receptacles indistinctly visible through chitin. Color in alcohol: Carapace, cheliceræ, palpi and first femora reddish-purple, coxæ and trochanters yellow; second, third and fourth femora yellow at base, purplish in distal half. Rest of legs reddish-brown. Abdomen light lemon-yellow with a grey band running around edge just behind pedicle and along each lung which is yellowish-brown; behind this band a pair of transverse grey bands the first of which is interrupted in middle where an orange, chitinized area lies in front of it. The second grey band has a median, anterior projection. Between the two bands, on the yellow field there are two pairs of little orange discs, the anterior pair being wider apart and twice as large as the posterior pair. Below, cheliceræ, palpi, maxillæ, lip and sternum dark reddish-brown. Coxæ and trochanters yellow. First femora purple. Second, third and fourth femora yellow at base, reddish-black beyond basal third. Rest of legs reddish-brown. Abdomen yellow, in front of genital fold yellowish-brown. Two rows of orange dots in mid-venter. Purple ring around spinnerets which are yellowish-brown. Abdomen above and below sparsely clothed with short, dark hair. Habitat: Panama, Guatemala and Mexico.

Family Ctenidæ

Ctenus W-notatus sp. nov. (Figures 79 and 80).

Female. Total with cheliceræ 15.0 mm. Carapace 6.80 mm. long, 5.61 mm. wide. Carapace high, thoracic groove longitudinal. Ratio of eyes AME : ALE : PME : PLE = 10 : 6.5 : 14 : 13. AME separated from each other by 7/10 of their diameter, from PME by 4/10 of their diameter, from anterior edge of carapace by their radius. ALE separated from AME by 11/10 of the diameter

of the latter, from PME by their diameter, from PLE by twice their diameter. PME separated from each other by their radius, from PLE by 16/13 of their diameter. PLE are separated from each other by four of their diameters. Quadrangle wider than long in ratio 32:28, wider behind than in front in ratio 32:24.

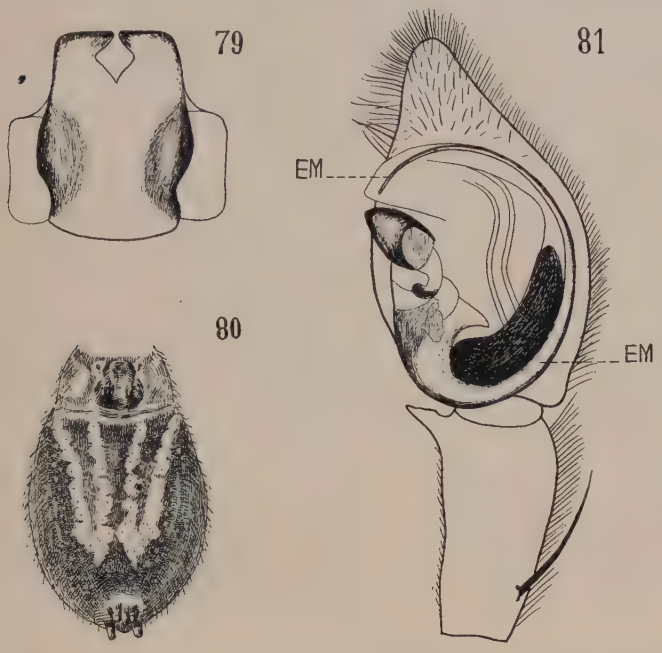


FIGURE 79, **Ctenus W-notatus** sp. nov., epigynum. FIGURE 80, Same, ventral view of abdomen. FIGURE 81, *Ctenus sinuatipes* F. Cambridge, left palp of male.

Arrangement of bristles on carapace irregular; generally speaking they form a recurved arc around eyegroup with other bristles present between eyes. Carapace clothed with short black and short white hair. *Chelicerœ* with powerful boss, anteriorly clothed with black bristles and white hair. Promargin with three teeth of which median is strongest, retromargin with seven teeth of which first and second are quite small, third half as large as fourth which is of the same size with the fifth, sixth and seventh. Scopula on promargin only, yellowish-brown. A soft brush of hair on lower surface of chelicerœ behind teeth of retromargin. *Lip* longer than

wide in ratio 38 : 30, emarginate at tip, with long basal excavations on sides which make the lip look longer than it really is. *Maxillæ* with inner edge straight, outer edge distinctly concave. Scopula yellowish-brown. Serrula very short, black. Sternum longer than wide in ratio 35 : 30, truncated in front, pointed behind. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	4.760	6.800	3.625	1.360	16.545
II	4.675	6.120	3.570	1.360	15.725
III	4.080	5.100	3.485	1.275	13.940
IV	5.270	6.800	6.120	1.785	19.975

All tarsi scopulate to base. Scopula of fourth tarsus light and longitudinally bisected by a band of setæ. Other scopulæ entire. First and second metatarsi lightly scopulate to base. Claw tufts on all tarsi. Trichobothria numerous on all tibiæ, metatarsi and tarsi, in two rows. Claws with two teeth. Palpal claw with four teeth of which the third and fourth are as large as terminal portion of claw.

Spines. First leg. Femur—dorsal 1-1-1, prolateral 0-2-1, retrolateral 1-1-0, ventral 0. Patella 0. Tibia—ventral 2-2-2-2-2, elsewhere 0. Metatarsus—ventral 2-2-2, elsewhere 0. *Second leg* same as first, except: Femur—prolateral 1-1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1-1-1, retrolateral 1-1-0-1, ventral 0. Patella—prolateral 1, retrolateral 1, elsewhere 0. Tibia—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 1 in middle, prolateral 1-1-2, retrolateral 1-1-2, ventral 2-2-2. *Fourth leg* same as third, except: Femur retrolateral 0-0-1. *Palp.* Femur—dorsal 1-1-1, prolateral 1 apical, retrolateral 1 apical, ventral 0. Patella—prolateral 1, elsewhere 0. Tibia—dorsal 1, prolateral 2, retrolateral 1, ventral 0. Terminal joint—dorsal 0, prolateral 2-1, retrolateral 1-1, ventral 0. Epigynum prominent, red, with a diamond-shaped median anterior excavation. Color in alcohol: Generally reddish-brown, with grey abdomen. Carapace with a light, middorsal band. Abdomen with a distinct W-mark below, extending from genital fold almost to spinnerets. Habitat: Wilcox camp on San Lorenzo River.

Ctenus sinuatipes F. Cambridge (Figure 81).

The male of this species has been described and figured by F. Cambridge. However, his figure of the palp is rather poor, so

that a new figure made from a specimen which unquestionably belongs to this species may not be out of place. I have a specimen from Cerro Flores and another and much larger one from Culebra. There are no appreciable differences in any structures of the two specimens.

Family Agelenidæ

Agelena panamana sp. nov. (Figure 82).

Female. Total 10.0 mm. Carapace 4.6 mm. long, 3.1 mm. wide. Both rows of eyes strongly procurved. Ratio of eyes AME :

Figure 82, **Agelena panamana** sp. nov., epigynum of female.

ALE : PME : PLE = 5 : 6 : 5 : 5. AME separated from each other by 3/5 of their diameter and by the same distance from ALE. Eyes of posterior row equidistant, separated from each other by their diameter. Quadrangle wider than long in ratio 16 : 13, wider behind than in front. Laterals separated by less than radius of PLE. AME separated from edge of carapace by twice their diameter, ALE by their diameter. *Chelicerœ* with prominent yellow boss, geniculate at base. Promargin with 3 teeth, retromargin with 5 teeth (on right chelicera with 6 teeth). Scopula on promargin only. *Lip* wider than long in ratio 23 : 18, articulated to maxillæ near base. *Maxillæ* almost parallel, twice as long as lip, not quite twice as long as wide, with short, brown scopula and long serrula. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	4.420	5.185	4.080	2.125	15.810
II	4.250	4.420	3.570	1.785	14.025
III	4.250	4.420	3.570	1.700	13.940
IV	4.675	5.525	5.015	2.125	17.340

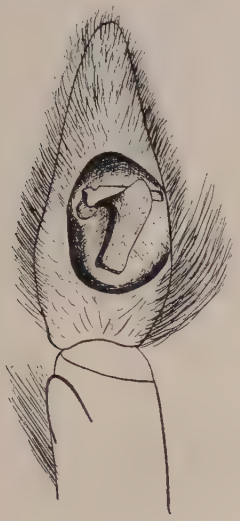
Spines. First leg. Femur—dorsal 1-1-1, prolateral 1-1-1, retrolateral 0-1-1, ventral 0. Patella—dorsal 1-1 bristles, elsewhere 0. Tibia—ventral 2-2-2, elsewhere 0. Metatarsus—dorsal 0, prolateral 1 in middle, retrolateral 0, ventral 2-2-3. *Second leg* same as first, except: Tibia—ventral 1-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1-1, retrolateral 0-1-1, ventral 0. Patella—dorsal 1-1 bristles, elsewhere 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 1-1-2. Metatarsus—dorsal 1-2-2, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-1. Tarsus—prolateral 1, retrolateral 1-1, elsewhere 0. *Fourth leg* same as third, except: Femur—retrolateral 0-0-1. Metatarsus—dorsal 1-1, prolateral 1-1-1, retrolateral 1-1-1, ventral 1-2-2-2. Upper claws on all tarsi with numerous teeth. Third claw with 3 teeth. Trichobothria in two rows on all tibiæ, in 1 row on all metatarsi and tarsi. Palp very spinose, especially terminal joint. Body and legs clothed with plumose hair. Sternum longer than wide in ratio 25 : 22, truncated in front, pointed behind between fourth coxæ which are separate. Terminal joint of posterior spinnerets considerably longer than basal joint, reddish. Coloration typical of the genus. Habitat: Wilcox camp on San Lorenzo River.

Family Pisauridæ

Ancylometes valentinei sp. nov. (Figures 83-85).

a) Female. Total with cheliceræ 19.0 mm. Carapace 9.52 mm. long, 8.16 mm. wide, rather unusually flat, posteriorly strongly emarginate; thoracic portion almost circular; head with parallel sides, 4.76 mm. wide. Thoracic groove longitudinal. Lateral eyes on joint tubercle which is prominent with a very steep declivity to the outside where its wall forms a right angle with the surface of the carapace. Arrangement of eyes as in *Ctenus*. Ratio of eyes AME : ALE : PME : PLE = 20 : 11 : 27 : 25. AME separated from each other by their radius, from PME by 9/20 of their diameter, from edge of clypeus by 12/20 of their diameter. PME separated from each other by 12/27 of their diameter, from PLE by their diameter. ALE separated from PME by 12/11 of their diameter and from PLE by twice their diameter. PLE separated from each other by four diameters. Quadrangle wider than long in ratio 66 : 57, wider behind than in front in ratio 66 : 46. Length of lateral ocular tubercle 1.53 mm. Carapace clothed with

short white hair, while the black lines extending to base of each leg are formed by short black hair. Behind and slightly to inside of each lateral tubercle a group of bristles with black base, but white toward end. A row of black bristles along anterior edge of carapace. Long white hair between eyes, on clypeus and on angles over the boss. *Chelicerœ* powerful, with red boss, clothed with long white bristles. Promargin with 3 teeth of which median



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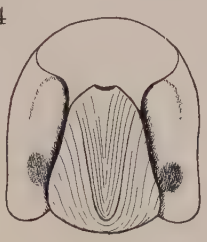

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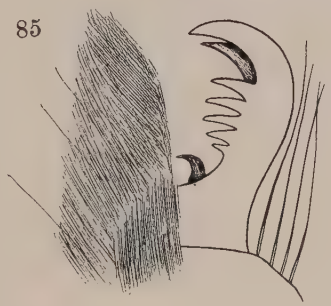

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FIGURE 83, **Ancylometes valentinei** sp. nov., right palp from below. FIGURE 84, Same, epigynum. FIGURE 85, Same, tarsal claws of female.

is strongest. Retromargin with 4 equidistant, equal teeth. Scopula on promargin only, yellowish-brown. *Lip* longer than wide in ratio 23:20, truncated at end, strongly excavated on sides at base, so that width of lip at base is only 13/23 of its length. At end of lip long, converging black hairs. *Maxillæ* very slightly concave on outside, so that the outer edges of the right and left maxilla appear to be parallel. Toward end outer edge is so strongly curved inward that the truncatures form between them an angle of only 50°. The truncatures are covered with a brown

scopula. *Sternum* a little longer than wide in ratio 48:45, slightly, but distinctly emarginate in front, pointed behind, clothed with long black hair which is lighter toward the margin of the sternum. Posterior coxæ contiguous, thickly clothed with yellow hair. The other coxæ are clothed with similar hair only in posterior half, while their anterior half is sparsely clothed with black hair. There is, however, no sharp demarkation line between the two kinds of hair. All trochanters strongly notched. *Legs* 2143, rather unusually long.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	10.3	14.2	9.6	3.2	37.3
II	10.7	14.0	9.5	3.2	37.4
III	9.1	11.1	8.0	2.8	31.0
IV	10.7	12.4	10.3	3.2	36.6

Heavy scopula on all tarsi, reaching to base, but leaving a bald diamond-shaped spot near the base. First, second and third metatarsi scopulate to base of proximal pair of ventral spines. Fourth metatarsi scopulate to middle. All metatarsal scopulæ have oblique troughs free from hair, for the reception of the heavy ventral spines.

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1-1, ventral 0. Patella—prolateral 1, retrolateral 1, elsewhere 0. Tibia—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2-2-2. Metatarsus—dorsal 0, prolateral 1-1-0, retrolateral 1-1-0, ventral 2-2-2-1. *Second leg* same as first. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1-1, retrolateral 1-1-1, ventral 0. Patella—prolateral 1, retrolateral 1, elsewhere 0. Tibia—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-1. *Fourth leg* same as third, except: Femur—retrolateral 0-0-1. Metatarsus—ventral 1 additional spine in retroventral row, so that there are three proventral, four retroventral and a midventral apical spine. *Palp.* Femur—dorsal 1-1-2, prolateral 1 at end, retrolateral 1 at end, ventral 0. Patella—prolateral 1, elsewhere 0. Tibia—dorsal 1 near base, prolateral 2, near base, elsewhere 0. Terminal joint—prolateral 2, retrolateral 1, elsewhere 0. No peculiar pad of hair on any joint. Palpal claw hidden by hair, with but two teeth. Upper claws on all tarsi similar, strongly curved, with 5 teeth. Third claw small, smooth. Ungual

tufts wanting, but scopulæ project beyond end of tarsus simulating true tufts. Epigynum red and concealed by yellow hair; external surface of central piece longitudinally fluted (Figure 84). Color in alcohol: Carapace red-brown with four pairs of radiating black lines. Legs and palpi red-brown. Abdomen dark grey, hairy. Cheliceræ reddish-black with three longitudinal bands of white hair. Color below same as above, except that lip and maxillæ are somewhat darker, though not as dark as cheliceræ. Venter of abdomen yellowish-grey.

b) Male. Total with cheliceræ 21.0 mm. Carapace 9.52 mm. long, 8.16 mm. wide in middle, 4.25 mm. wide at anterior margin which is truncated. Thoracic groove longitudinal. Ratio of eyes AME : ALE : PME : PLE = 20 : 12 : 28 : 28. Lateral eyes on tubercle as in female. Arrangement of eyes as in female. AME separated from each other by 8/20 of their diameter, from PME by 7/20 of their diameter, from ALE by 17/20 of their diameter, from edge of carapace by 13/20 of their diameter. ALE separated from PME by 9/12 of their diameter, from PLE by 16/12 of their diameter. PME separated from each other by 8/28 of their diameter, from PLE by 18/28 of their diameter. PLE separated from each other by three diameters. Quadrangle wider behind than in front in ratio 62 : 44, wider than long in ratio 62 : 52. Arrangement of bristles and hair same as in female, but lines slightly sharper. There is also an indistinct black line back of each PLE, the two slightly diverging as far as their middle, then just as slightly converging till they reach the cephalic sulcus. Central area of carapace darker. *Legs* longer and more slender than in the female, but in the same order 2143.

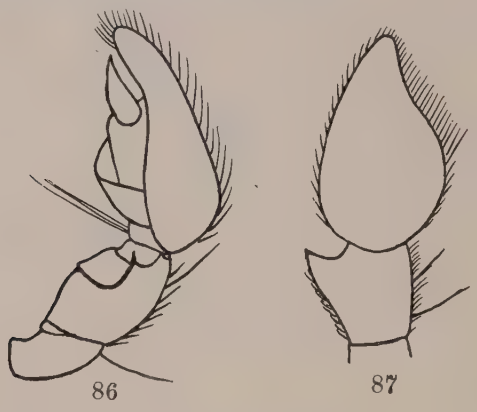
	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	12.4	17.3	13.3	4.7	47.7
II	13.0	17.2	13.5	4.4	48.1
III	11.0	13.0	11.0	4.0	39.0
IV	12.0	14.3	13.9	4.0	44.2

Arrangement of spines same as in female, except that there are on all tibiæ 1-1 dorsal spines, and no spines on the terminal joint of the palp. Trichobothria numerous on all tibiæ, metatarsi and tarsi. Upper claws with 7 teeth. Palp and tibial apophysis shown in Figure 83. Color of abdomen same as in female, but there is a pair of narrow, parallel longitudinal black lines marking

the heart and uniting in middle of dorsum. Venter altogether lighter than in the female. Habitat: Wilcox camp on San Lorenzo River.

Dyrines lineatipes sp. nov. (Figures 86 and 87).

Male. Total 2.55 mm. Carapace 1.615 mm. long, 1.445 mm. wide, convex, highest in posterior region with posterior declivity almost vertical. Thoracic groove longitudinal. Both rows of eyes recurved. Ratio of eyes AME : ALE : PME : PLE = 3 : 2 :



86 87

FIGURE 86, **Dyrines lineatipes** sp. nov., left palp in side view. FIGURE 87, Same, left palp from above.

4 : 4. AME separated from each other by their diameter, from ALE by 1/3 of their diameter. PME separated from each other by 3/4 of their diameter, from PLE by 5/4 of their diameter. Quadrangle wider behind than in front, wider than long in ratio 10 : 9. Clypeus equal to 1/3 of diameter of AME. Promargin of *cheliceræ* with four teeth of which only the third from base approaches in size those of retromargin, the other three teeth being quite small. Retromargin with 3 equal and equidistant teeth. Scopula on promargin only. *Lip* longer than wide in ratio 8 : 6, reaching a little beyond middle of maxillæ. *Maxillæ* wider at end, slightly converging, with scopula only on inner edge. *Sternum* triangular, longer than wide in ratio 25 : 22 broadly truncated in front, pointed behind between the hind coxæ. First pair of legs missing, so that the order of the legs remains uncertain.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	?	?	?	?	?
II	1.870	2.550	2.125	0.765	7.310
III	1.360	1.530	1.360	0.510	4.760
IV	2.125	2.380	2.550	0.765	7.720

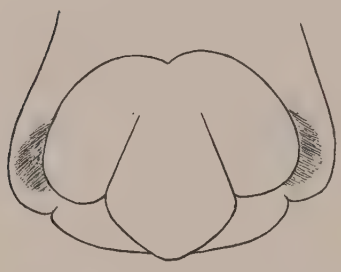
Spines. Second leg. Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 0-1 at end, ventral 0. Patella 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2-2-1. (Spines on metatarsus colored like hair and difficult to see.) Third and fourth legs same as first. Upper claws with 6 or 7 teeth, third claw with a single, thin, strongly curved long tooth. Several serrated bristles at end of tarsi. Trichobothria numerous on all tibiæ, metatarsi and tarsi. *Palp.* Femur—dorsal 1-1-1-2, retrolateral 2, elsewhere 0. Patella with a middorsal dark line, tibia with a middorsal and a retrolateral line. Embolus stout, screw-shaped. Color in alcohol: Carapace yellow, abdomen grey, cheliceræ, lip, maxillæ, sternum and coxæ yellow. Legs yellow with longitudinal black lines. There are 5 lines on femora (3 dorsal, 1 prolateral and 1 retrolateral), 3 lines on patellæ and tibiæ and 1 dorsal line on metatarsi and tarsi. The line on the metatarsi begins with a fork. The species is closely related to *D. striatipes* of Simon, of which the female alone is known. The relatively small size and the color of the carapace make it more probable that *D. lienatipes* is a distinct species. Habitat: Wilcox camp on San Lorenzo River.

Enna minor sp. nov. (Figure 88).

Female. Total with cheliceræ 8.0 mm. Carapace 3.400 mm. long, 2.975 mm. wide in middle, 1.785 mm. wide at anterior margin. Thoracic groove longitudinal. Anterior row of eyes slightly recurved, as long as the space occupied by PME. Posterior row recurved. Ratio of eyes AME : ALE : PME : PLE = 5 : 4 : 7 : 8. AME separated from each other by 4/5 of their diameter, from ALE by 2/5 of their diameter. PME separated from each other by 10/7 of their diameter, from PLE by 4/7 of their diameter. Quadrangle narrower in front than behind, much broader than long in ratio 24 : 15. Clypeus equal to two diameters of AME. Promargin of *cheliceræ* with 3 teeth of which the median is strongest. Retromargin with 4 teeth on the right, but only 3

teeth on the left chelicera. Scopula on promargin only. *Lip* as long as wide, excavated on sides at base, transversely truncated at end, with sides parallel. *Maxillæ* with thick scopula, but without serrula, wider at end than at base and parallel to each other.

88



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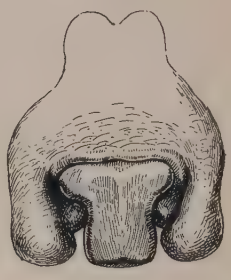


FIGURE 88, **Enna minor** spo nov., epigynum. FIGURE 89, **Trechalea magnifica** sp. nov., epigynum.

Sternum as wide as long, broadly truncated in front, pointed behind, sparsely clothed with long bristles. All trochanters notched. Tarsi straight, not flexible, lightly scopulate to base. Metatarsi not scopulate. Upper claws with several teeth, third claw with a single tooth. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	3.740	4.930	3.400	1.530	13.600
II	3.825	4.930	3.230	1.360	13.345
III	3.060	3.400	2.720	1.105	10.285
IV	3.655	4.250	3.740	1.360	13.005

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1-1-1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 bristles, retrolateral 1, elsewhere 0. Tibia—dorsal 1-1 bristle, prolateral 1-1, retrolateral 1-1, ventral 2-2-2-2 (fourth pair small). Metatarsus —dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-1. *Second leg* same as first, except: Femur—retrolateral 1-1-1-1. *Third leg* same as first, except: Femur—retrolateral 1-1. *Fourth leg* same as first, except: Femur—dorsal 1-1-1-1, prolateral 1-1, retrolateral 1. Epigynum concealed by hair. When fully exposed it appears trilobate as in Figure 88. Habitat: Santiago.

Trechalea magnifica sp. nov. (Figure 89).

Female. Total without cheliceræ 21.5 mm., with cheliceræ 25.0 mm. Carapace 9.8 mm. long, 10.0 mm. wide in middle, 5.0 mm. wide at anterior margin. Thoracic groove longitudinal, long and narrow. Entire eyegroup set in black, the black area having the shape of a crescent with concavity directed backward. Clypeus very slightly inclined, 14/5 of the diameter of AME. Anterior row of eyes recurved, a fraction longer than space occupied by PME. Posterior row strongly recurved and longer than anterior row in ratio 39:21. Ratio of eyes AME:ALE:PME:PLE = 5:3.5:8:9. AME separated from each other by 3/5 of their diameter, from ALE by half of their radius. PME separated from each other by their radius, from PLE by their diameter. Quadrangle much narrower in front than behind, a little wider than long in ratio 19:18. Behind PLE on each side of carapace a group of black, stout bristles curved forward. Anterior edge of carapace with grey hair. Carapace clothed with short black hair. *Cheliceræ* long, directed forward, clothed with short, recumbent grey hair and long dark, erect hair. Promargin with 3 teeth of which median is strongest. Retromargin with 3 strong teeth, equal in size but not equally spaced, the median tooth being nearer to the distal one. Scopula on promargin only. *Lip* about as long as wide (ratio 23 long, 22 wide), slightly narrowed toward base. *Maxillæ* parallel to each other, wider in front than at base, with strong scopula. *Sternum* longer than wide in ratio 61:56, broadly truncated in front, produced as a long, pointed tongue between hind coxæ. If this portion of sternum be disregarded, then sternum is wider than long in ratio 45:37. *Legs* 4213.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	15.5	20.0	14.6	9.0	59.1
II	18.0	22.0	16.6	10.0	66.6
III	14.2	16.3	13.0	8.0	51.5
IV	19.0	20.6	20.6	12.0	72.2

Spines. First leg. Femur—dorsal 1-1-1, distal spine very small; prolateral 1-1-1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1 at end, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2-2 last pair small. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-1. *Second leg* same as first. *Third leg.* Femur—

dorsal 1-1-1, distal spine very small; prolateral 1-1-1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1 at end, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-1. *Fourth leg* same as third, except: Femur—dorsal 1-0-1, prolateral 1-1-0-1, retrolateral 0-0-1. Metatarsus with 13 ventral spines which are not in pairs and of which three small spines are apical in position. *Palp.* Femur—dorsal 1-1-1-2, prolateral 1, retrolateral 1, ventral 0. Patella—dorsal 1-1, prolateral 1, elsewhere 0. Tibia—dorsal 2 near base, prolateral 1-1, retrolateral 1. Elsewhere 0. All trochanters deeply notched. All tarsi flexible. All tarsi and metatarsi scopulate to base. Upper claws with from 4 to 6 teeth. Third claw smooth. Palpal claw toothed. Epigynum completely concealed by grey hair. When exposed it appears in the shape of a rectangle with a central T-tongue. Color in alcohol: Carapace yellow with eyearea black and a dark marginal line. Also indistinct dark markings on sulci. Cheliceræ black. Abdomen dark greyish-brown with a light median mark and a light, anterior, transverse band. Legs and palpi above yellow spotted with black. Metatarsi and tarsi almost entirely black. Lip and maxillæ dark brown. Sternum and coxæ yellow. Trochanters red-brown toward end. Femora yellow, darker toward end. Rest of legs dark. Abdomen yellow below. Spinnerets dark brown. Epigynum red.

This species is closely related to *T. longitarsis* C. Koch, but the description given by F. Cambridge for the latter species shows that *T. magnifica* is a distinct species. For example, the clypeus in *T. longitarsis* is "6 times as high as the diameter of an anterior central eye (in male four times)," whereas in *T. magnifica* it is only 14/5, *i. e.* not quite three diameters as high. Again, *T. longitarsis* is described as having the "central posterior eyes one diameter apart," while in *T. magnifica* they are separated by only their radius. Habitat: Wilcox camp on San Lorenzo River, Bocas del Monte, La Mesa and Santiago.

Family Oxyopidæ

Peucetia rubricapilla sp. nov. (Figures 90-92).

Female. Total without cheliceræ 8.5 mm. Carapace 3.23 mm. long, 2.72 mm. wide in middle, much narrowed anteriorly, where

it measures only 1.615 mm. in width and has parallel sides. Thoracic groove longitudinal. Head very high. Angle formed by thoracic midline and clypeus measures 90°. Clypeus 0.738 mm. high, *i. e.* is almost as high as the entire eyegroup. Eyegroup characteristic of the genus. Its width is greater than its length

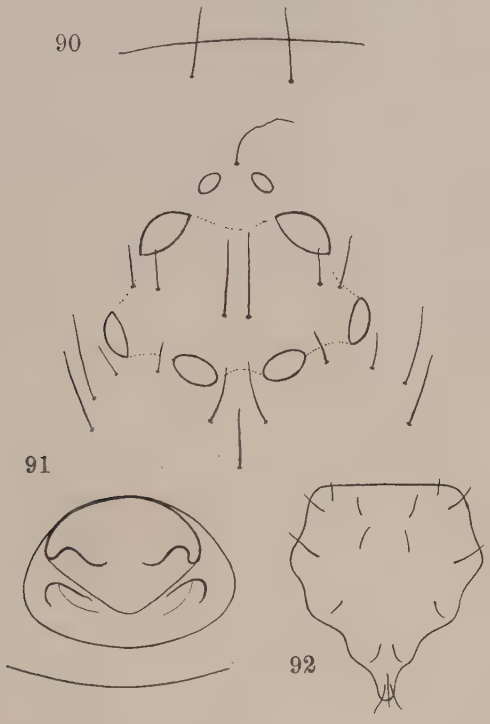


FIGURE 90, **Peucetia rubricapilla** sp. nov., eyegroup with clypeus, showing arrangement of bristles. FIGURE 91, Same, epigynum. FIGURE 92, Same, sternum showing arrangement of bristles.

in ratio 28:22.5. Ratio of eyes AME:ALE:PME:PLE = 3:7:5:5. AME separated from each other by their diameter and from ALE by but little less. ALE separated from each other by 8/7 of their diameter, from PLE by 6/7 of their diameter. PME separated from each other and from PLE by their diameter. Space between AME and PME clothed with white hair. Space between second and third row of eyes clothed with red hair. Arrangement of bristles very definite. One bristle is immediately

in front of AME. One pair of long bristles is in front of the eyegroup and another pair of long bristles in the middle of the eyegroup. One bristle on each side between PLE and PME. A row of 3 bristles on each side behind PLE. One pair of bristles behind PME, followed by 1-1-2 bristles (the pair near thoracic groove). Two rows of small hairs in median line behind eyegroup extend to thoracic groove and a similar row of hair in each cephalothoracic sulcus which separates head from thorax. Edge of carapace over boss on each side quite black. *Cheliceræ* as long as face, with green boss. Margins very short, smooth. Small scopula on promargin, none on retromargin. *Lip* much longer than wide, gradually narrowed toward end which is emarginate. It reaches beyond middle of maxillæ. *Sternum* green, triangular, longer than wide in ratio 44:38, broadly truncated in front, pointed behind. Arrangement of bristles on sternum very definite and is shown in Figure 92. *Maxillæ* very long and narrow, with dark scopula and black serrula. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	4.420	5.440	4.080	2.380	16.320
II	4.420	4.930	3.910	1.785	15.045
III	3.655	3.995	3.230	0.935	11.815
IV	4.080	4.335	3.655	1.020	13.090

Fourth coxæ separated by somewhat less than their diameter. All trochanters notched. Upper claws with 5 to 6 teeth. Third claw with a single tooth. Four true serrated bristles present on tarsi. Trichobothria numerous. Over a dozen at base and 2 in middle of tibiæ; a row of short trichobothria on metatarsi and tarsi. All spines and some hairs surrounded by black.

Spines. The arrangement on all legs is the same and is as follows: Femur—spines—dorsal 1-1-1, prolateral 1-1-1, retrolateral 1-1-1, ventral 0; hairs—prolateral a row of from 8-9, retrolateral a row of from 8 to 9, ventral 9 pairs. Patella—spines—dorsal 1-1, retrolateral 1, elsewhere 0. Tibia—spines—dorsal 1-1, prolateral 1, retrolateral 1, ventral 2-2-2. Metatarsus—spines—dorsal 2-2-2; ventral 2-2-2, elsewhere 0. Colulus well developed. Epigynum transversely oval. Color in alcohol: Carapace light green, except between the eyes where it is red. Abdomen green. Cheliceræ yellow. Legs yellow with black spots, in places with a tinge of carmine. Maxillæ, lip and sternum green. Spinnerets

yellow. Epigynum yellowish-green with a narrow black line along the edge of the fossa. Palp with many spines on all segments. Palpal claw toothed. Habitat: Sona.

Family Lycosidæ

Key to American Genera of Lycosidæ

1. Eyes in two rows, all of about the same size ..... *Rhoicinus.*
   *Eyes in three rows, those of the first row much the smallest 2
2. Retromargin of cheliceræ with 4 teeth 3
   *Retromargin with 2 or 3 teeth 5
3. First row of eyes recurved. Anterior spinnerets two-jointed ..... *Teippus.*
   *First row not recurved. Anterior spinnerets with a single joint 4
4. Eyes of first row nearly equidistant. First row shorter than third ..... *Sosippus.*
   *AME closer to each other than to ALE. First row wider than third ..... *Porrima.*
5. Anterior spinnerets much longer than posterior ones ..... *Moenkhousiana.*
   *Not so 6
6. Anterior tibiæ with 2 or 3 pairs of spines below 7
   *With five pairs of very long spines below . . *Sosilaus.*
7. Head with vertical sides 8
   *Head with slanting sides or else transverse plate of epigynum with forked ends or embolus elbowed 11
8. Posterior spinnerets much longer than anterior ones ..... *Aulonia.*
   *Posterior spinnerets not much longer than anterior ones 9
9. Clypeus high, not lower than quadrangle . . *Algidus.*
   *Clypeus low, never as high as quadrangle 10
10. First row of eyes strongly procurved. AME much nearer to each other than to ALE ..... *Trabea.*
    *First row not strongly procurved, its eyes fairly equidistant ..... *Pardosa.*
11. Retromargin of cheliceræ with 2 teeth . . *Alopecosa.*
    *Retromargin with 3 teeth 12

12. Promargin with 2 teeth. Carapace glabrous *Allocosa.*
*Promargin with 3 teeth, rarely with 2. Carapace not glabrous 13

13. Posterior spinnerets longer than anterior ones .. 14
*Posterior spinnerets not distinctly longer 15

14. First row of eyes not or but little wider than second. Distal pair of ventral spines on anterior tibiæ never apical. Carapace with a V-shaped mark enclosed in the median pale band .....*Pirata.*
*First row much wider than second ....*Diapontia.*

15. A middorsal spine on third and fourth tibiæ near base 17
*Either the spine is wanting or there is a bristle in its place 16

16. Middorsal spines on third and fourth tibiæ wanting .....*Geolycosa.*
*A bristle in place of basal middorsal spine on third and fourth tibiæ. Second middorsal spine as usual .....*Arctosa.*

17. Tarsi not scopulate. First row of eyes strongly procurved .....*Orinocosa.*
*At least anterior tarsi scopulate 18

18. Transverse arm of epigynum with forked ends. Embolus elbowed .....*Schizocosa.*
*Transverse arm with solid ends. Embolus curved .....*Lycosa.*

Pardosa albopilosa sp. nov. (Figure 93).

Male. Total 6.120 mm. Carapace 3.230 mm. long, 2.550 mm. wide. Head as high as thorax, so that thoracic groove, which is longitudinal, is on the same height with the region of the PLE. Anteriorly carapace slopes gently to the eyes of the second row and thence becomes almost vertical. Posteriorly to thoracic groove carapace slopes downward at an angle of 60°. Sides of face steep, though not quite vertical. Viewed from in front, anterior row of eyes is curved downward and shorter than second row in ratio 20:27. Ratio of eyes AME:ALE:PME:PLE = 5:3.5:12:9.5. AME separated from each other by 2/5 of their diameter, from ALE by 1/5 of their diameter and from PME by 3/5 of their diameter. PLE are wider apart than PME in ratio 35:27 and the quadrangle of the posteriors is wider behind than long in ratio

35 : 27. Clypeus but little higher than diameter of AME. Carapace clothed with short black hair on sides, with a fringe of short white hair on margins, with a few white hairs on median yellow band behind thoracic groove, with short white and long black hair between the eyes. Elsewhere carapace is devoid of hair. There

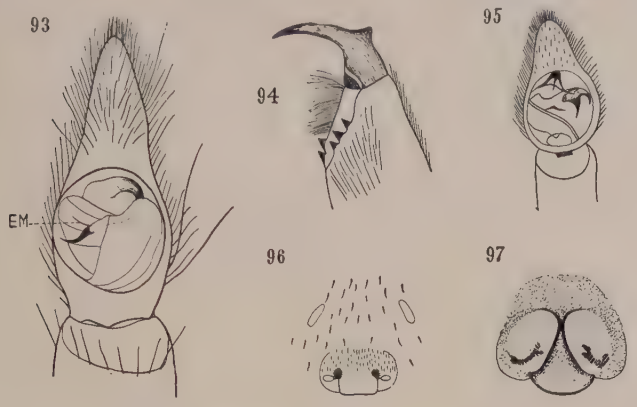


FIGURE 93, **Pardosa albopilosa** sp. nov., right palp. FIGURE 94, **Arctosa cheluncata** sp. nov., end of left chelicera from below, showing hook at base of fang. FIGURE 95, Same, left palp from below. FIGURE 96, Same, epigynum and ♂ pair of muscular attachments in front of it. FIGURE 97, **Arctosa panamana** sp. nov., epigynum.

is also a single long black bristle between AME. *Cheliceræ* with boss. Promargin with 3 teeth, of which the median is largest. Retromargin with three equal teeth. Scopula on promargin only. A brush of rather long hair on underside of cheliceræ. *Lip* wider than long in ratio 8 : 6, considerably narrowed at base, slightly emarginate at tip, with a few black bristles. *Maxillæ* almost parallel, with well-developed brown scopula and serrula. *Sternum* much longer than wide in ratio 40 : 32, truncated in front, pointed behind, clothed with black hair. Posterior coxæ contiguous. All trochanters notched. *Legs* 4132.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.550	3.145	1.955	1.105	8.755
II	2.125	2.720	1.955	1.105	7.905
III	2.125	2.635	2.210	1.105	8.075
IV	2.975	3.400	3.855	1.530	11.760

All tarsi slightly scopulate to base.

Spines. First leg. Femur—dorsal 1-1-1-2, prolateral 2 at end; retrolateral 0, ventral 0. Patella 0. Tibia—dorsal 1-1 fine bristles; prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus —dorsal 0, prolateral 1-1-1, retrolateral 1-1, ventral 2-2-2-1. *Second leg* same as first. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1, the first a bristle; prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus —dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-1. *Fourth leg* same as third, except: Femur—retrolateral 0-0-1. Upper claws on all legs with 4 teeth, third claw smooth. Trichobothria in two rows on all tibiæ, metatarsi and tarsi. Tibia of palp a little stouter than terminal joint. Cymbium with seven spines at end. Embolus long, curved. Color in alcohol: Carapace black with a yellow longitudinal median band beginning half way between eyes of second and third row, suddenly widening behind the latter to a little more than the space between the eyes, forming lateral indentations near rear end of head and then gradually attenuating toward thoracic groove. Yellow color of band due entirely to the chitin and not to hair. On the other hand the black color on the sides of the carapace is partly due to pigmentation of the chitin and partly to black hair. Abdomen above with a broad yellow longitudinal median band and brown sides. Heart outlined by dark mottling and there are at the end of the heart two triangular marks, one behind the other, with their apices directed forward. Hair on dorsum of abdomen black except on small patch in front of heart, where it is white and long. All trochanters yellow above. Femur of palp and of first and second leg black. Third and fourth femora with four indistinct transverse black marks, otherwise yellow. Patella of palp dark, though not black, tibia also dark at base, but becoming yellow toward end, terminal joint yellow. First and second patellæ and proximal half of tibiæ are black; but from here on to the end of the tarsi first and second legs are white owing to the color of the hair. Spines black. Hair on black parts of legs black. Third and fourth tibia, metatarsus and tarsus yellow above, without markings, but the hair on them is black. Face

black. Chelicerae in front very dark brown with yellow on inside, light brown below. Lip dark brown with yellow tip. Maxillæ also light brown with yellow tip. Sternum, first and second coxæ dark brown, third coxæ yellow with black along anterior edge, fourth coxæ yellow. Femur, patella and tibia of palp uniformly dark below. All trochanters yellow, those of first and second pair with dark sides. First and second femora, patellæ and proximal half of tibiæ black below, rest of first leg below yellow with white hair, of second leg yellow with black hair. Third and fourth legs entirely yellow below with black hair. Abdomen greyish-yellow below with darker median stripe. Spinnerets yellow with black hair. Habitat: Panama City.

Arctosa cheluncata sp. nov. (Figures 94-96).

a) Male. Total without chelicerae 5.780 mm. Carapace 3.400 mm. long, 2.465 mm. wide in middle, only half as wide in front. Thoracic groove longitudinal. Carapace clothed with short, recumbent, black hair. A few long black hairs in the eye-region and also in a median line behind eyes extending to thoracic groove. Posterior margin with black hair except in middle where there is no hair at all. Head with slanting sides. First row of eyes straight, slightly shorter than second in ratio 19:21. Ratio of eyes AME:ALE:PME:PLE = 4:3.4:9:7.5. AME separated from each other by their radius, from ALE by less than radius, from PME by their radius. PME separated from each other by their radius, from PLE by 2/3 of their diameter. PLE separated from each other by twice their diameter. Quadrangle of medians twice as wide behind as in front, wider behind than long in ratio 20:15. Quadrangle of posteriors wider behind than in front in ratio 25:20, and wider behind than long in the same ratio. Clypeus almost as high as diameter of AME, with four bristles in a transverse row. *Chelicerœ* comparatively slender, with rather long margins. Promargin with 2 teeth, retromargin with 3 teeth. Fang with a hook on outside (Figure 94). Scopula on promargin only. Lower surface of chelicerae clothed with long hair to base. Boss present, but not prominent. Carapace with a dark ridge in the places above the boss. *Lip* barely longer than wide, emarginate at tip, articulated to maxillæ one third from base. At end of lip long, black hair. *Maxillœ* with almost parallel sides,

slightly inclined over lip, with heavy scopula. *Sternum* subrotund, as long as wide, rounded behind in front of hind coxæ which are contiguous. Sternum sparsely clothed with long black hair. All trochanters deeply notched. Spines comparatively long, especially middorsal femoral and those on the metatarsi of third and fourth leg. Trichobothria on all tibiæ, metatarsi and tarsi. First and second tarsi slightly scopulate, third and fourth setose. Upper claws on first and second leg with 4 teeth, on third and fourth with 8 teeth. Third claw well developed, smooth. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.380	2.990	1.955	1.275	8.600
II	2.125	2.550	1.870	1.190	7.735
III	1.955	2.295	2.040	1.105	7.395
IV	2.550	3.485	3.230	1.530	10.795

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1, retrolateral 1-1, ventral 0. Patella—dorsal 1-1 fine bristles, prolateral 1, retrolateral 1, elsewhere 0. Tibia—dorsal 1 fine bristle near base, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-1. *Second leg* same as first, except: Femur—prolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1, ventral 0. Patella—dorsal 1-1 fine bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, first a bristle, second a spine situated beyond middle, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-1. *Fourth leg* same as third, except: Femur—retrolateral 0-1. *Palp.* Femur—dorsal 1-1-2, prolateral 1 at end, retrolateral 1 at end, ventral 0. Patella—prolateral 1, elsewhere 0. Spines on tibia cannot be distinguished from bristles. Structure of bulb shown in Figure 95. Color in alcohol: Carapace yellow mottled with grey. Eyeregion almost black. Legs yellow spotted above only with brown. Palp yellow except terminal joint which is black. Abdomen above brown with an indistinct middorsal yellow area and long black hair in front. Below entire spider yellow with spinnerets somewhat darker.

b) Female. Total without cheliceræ 9.180 mm. Carapace 3.995 mm. long, 3.400 mm. wide. Arrangement of eyes and of spines the same as in male. Cheliceræ more robust, with well-developed yellow boss. Fang without outer hook, evenly curved.

Colulus distinct, though small. Spinnerets equal in size. *Legs* 4132.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	2.720	3.230	2.040	1.360	9.350
II	2.245	2.890	1.955	1.190	8.280
III	2.245	2.635	2.245	1.190	8.315
IV	3.145	3.825	3.570	1.445	11.985

Coloration much as in male, but markings on carapace more definite and spots on legs more pronounced. Epigynum with a transversely rectangular flap (Figure 96). Habitat: Santiago, Remedios, La Mesa and Wilcox camp on San Lorenzo River.

Arctosa panamana sp. nov. (Figure 97).

Female. Total without cheliceræ 18.5 mm. Carapace 8.2 mm. long, 6.6 mm. wide. Thoracic groove longitudinal. Edge of carapace with long black and short white hair. Surface of carapace with short black and more scattered long black hair. Long black and short white hair on eyegroup. Head with slanting sides. First row of eyes straight, slightly shorter than second in ratio 40:45. Ratio of eyes AME:ALE:PME:PLE = 10:7:18:15. AME separated from each other by their radius, from ALE by 3/10 of their diameter, from PME by their radius. PME separated from each other by 11/18 of their diameter, from PLE by 13/18 of their diameter. Quadrangle of medians wider than long in ratio 42:30, much narrower in front than behind. Quadrangle of posteriors wider behind than in front, wider behind than long in ratio 52:42. Clypeus equal to 7/10 diameter of AME except in front of these eyes where it is equal to the diameter. Four long black bristles in a transverse row on clypeus, two pairs of similar bristles on the two sclerites between the clypeus and the cheliceræ. *Cheliceræ* with red boss and black bristles. Both margins with 3 teeth. On promargin median tooth largest, on retromargin distal tooth largest. Scopula on both margins. *Lip* much longer than wide in ratio 36:27, articulated to maxillæ one third from base, emarginate at tip. Long black hairs at end of lip. *Maxillæ* wider at end, almost parallel, with heavy scopula of brown hair. Surface clothed with very short black and very long black hair. *Sternum* as long as wide, evenly rounded in front, narrowed posteriorly, clothed with long black hair which extends also in a narrow line behind the sternum to base of petiolus.

Legs clothed with similar hair interspersed with fine short hair. Spines on anterior legs short, on posterior legs longer. First and second tarsi, metatarsi and tibiæ scopulate, the scopulæ entire. On third leg only tarsus and metatarsus scopulate. The metatarsal scopula entire, the tarsal scopula bisected longitudinally by a band of stiff hairs. Fourth tarsus and metatarsus also scopulate, but the scopula on metatarsus rather light and longitudinally bisected by a broad band of bristles. The fourth tarsal scopula also bisected by a broad band of hair. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	6.6	8.0	5.6	3.0	23.2
II	6.1	7.6	5.2	3.0	21.9
III	6.0	6.4	6.0	3.0	21.4
IV	7.5	9.8	8.5	4.1	29.9

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1-1 ventral 0. Patella—prolateral 1, retrolateral 1, elsewhere 0. Tibia—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-1. *Second leg* same as first, except: Femur—prolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1, ventral 0. Patella—prolateral 1, retrolateral 1, elsewhere 0. Tibia—dorsal 1-1, the first a bristle near base, the second a spine slightly beyond middle, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-2-1. *Fourth leg* same as third, except: Femur—prolateral 1-1, retrolateral 0-1. *Palp.* Femur—dorsal 1-1-2, prolateral 1 apical, retrolateral 1 apical, ventral 0. Patella—dorsal 1, prolateral 1, elsewhere 0. Tibia—dorsal 0, prolateral 2 near base, elsewhere 0. Terminal joint—prolateral 3 at base, elsewhere 0. Upper claws with 5 teeth on first and second pair of legs, with 11 teeth on third and fourth pair. Third claw almost rudimentary. Palpal claw with 2 teeth at base. Colulus very small. Epigynum as shown in Figure 97. Color in alcohol: Carapace yellow mottled with brown. Cheliceræ black with red boss. Lip and maxillæ reddish-brown with pale yellow ends. Sternum, abdomen and legs yellow below. Above, legs yellow spotted with brown, abdomen greyish-yellow. Spinnerets brown. Eggcocoon rather small. Habitat: Remedios.

KEY TO SPECIES OF LYCOSA FOUND IN PANAMA

1. Carapace longer than fourth Patella + Tibia 2
   *Carapace shorter than fourth Patella + Tibia .. 3
2. Metatarsi of second and third leg equally long
   L. dilatata F. Cambridge.
   *Third metatarsus longer than second
   L. pugnatrix (Keyserling).
3. Metatarsi of second and third leg equally long .. 4
   *Third metatarsus longer than second 5
4. Smaller species, about 13 mm. long. Abdomen above greyish-brown with anterior black spot in middle .....*L. fusca* (Keyserling).
   *Larger species, about 21 mm. or more. Abdomen above with a broad blackish median band extending to spinnerets .....*L. rabida* Walckenaer.
5. Clypeus less than radius of AME
   L. cerrofloresiana sp. nov.
   *Clypeus equal to diameter of AME 6
6. First and second metatarsi with one apical prolateral, one apical retrolateral and 2-2-1 ventral spines .....*L. longitarsis* F. Cambridge.
   * First and second metatarsi without lateral spines and second metatarsus with 2-2-3 ventral spines.
   L. sanlorenziana sp. nov.

Lycosa cerrofloresiana sp. nov. (Figure 98).

Male. Total with cheliceræ 7.8 mm. Carapace 4.420 mm. long, 3.400 mm. wide. Thoracic groove longitudinal. Head with slanting sides. First row of eyes shorter than second in ratio 31:40, slightly curved downward. Ratio of eyes AME:ALE:PME:PLE = 8:6:18:14. AME separated from each other by 2/8 of their diameter, from ALE by the same distance, from PME by 3/8 of their diameter. PME separated from each other by 8/18 of their diameter. PLE separated from each other by twice their diameter and from PME by their diameter. Quadrangle of posteriors wider behind than long in ratio 50:38. Clypeus 2.5/8 of the diameter of AME. On clypeus four bristles in a transverse row. Middorsal row of black bristles extends from behind eyegroup to thoracic groove, ending in a pair. A few

black bristles between posterior eyes and a row of black bristles on each side of eyegroup extending from cephalothoracic sulcus to edge of carapace. Eyes fringed with white hair toward the inside. *Chelicerœ* with red boss. Promargin with 3 teeth of

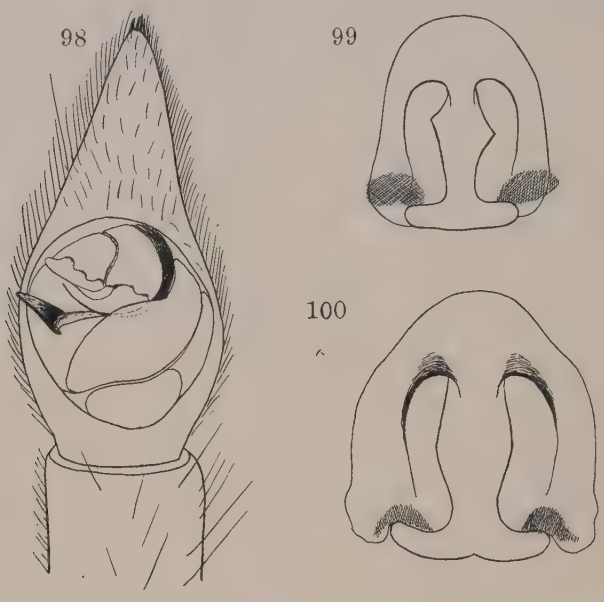


FIGURE 98, **Lycosa cerrofloresiana** sp. nov., right palp from below. FIGURE 99, **Lycosa sanlorenziana** sp. nov., epigynum. FIGURE 100, *Lycosa longitarsis* F. Cambridge, epigynum.

which the median is largest and the distal is smallest. Retromargin with 3 equal teeth. Scopula on promargin only. *Lip* as long as wide. *Maxillæ* with brown scopula and black serrula. *Sternum* longer than wide in ratio 22:18, truncated in front, pointed behind, clothed with black bristles. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	3.655	4.760	3.400	2.125	13.940
II	3.145	3.995	2.890	1.870	11.900
III	3.145	3.400	3.060	1.615	11.220
IV	4.250	4.845	4.590	2.125	15.810

All tarsi slightly scopulate. Anterior two pairs of scopulæ entire, posterior two pairs bisected longitudinally by a band of setæ. First metatarsi with light scopula in anterior third, other

metatarsi without scopula. Anterior tarsi distinctly curved. Upper claws on first and second tarsi with 4 teeth, on third and fourth tarsi with 7 teeth. Third claw smooth.

Spines long, especially the middorsal ones on femora. *First leg.* Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 fine bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1 fine bristles, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-3. *Second leg* same as first, except: Femur—prolateral 0-1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 0-1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 fine bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1 spines, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-3. *Fourth leg* same as third, except: Femur—retrolateral 0-0-1. Tibia—ventral 1-2-2-3. *Palp.* Femur—dorsal 1-1-2, prolateral 1 near end, retrolateral 1 near end, ventral 0. Patella—dorsal 1-1 fine bristles, prolateral 1, elsewhere 0. Tibia—dorsal 2, prolateral 1, elsewhere 0. Terminal joint—4 spines at end of cymbium. Trichobothria in two rows on all tibiæ and tarsi, in one row on all metatarsi. Palp shown in Figure 98. Color in alcohol: Carapace brown with a yellow median band of the typical Greek Φ type, beginning halfway between PME and PLE, narrowing gradually as far as PLE, then suddenly widening to form the arms of the letter Φ, then just as suddenly constricted and gradually attenuated until it reaches posterior end of carapace. A very fine marginal black line and an indistinct broad submarginal yellowish-brown band. Space between PME and, on each side of yellow band, between PME and PLE, black. Legs yellow, indistinctly spotted with brown. Abdomen brown above with 2 triangular black spots in front, enclosing end of white triangular mark which extends to middle of abdomen and in its turn encloses the dark spot over the heart. In the posterior half of the abdomen there are three transverse black marks with an angular projection in middle. Cheliceræ black on outer surface, reddish-brown on ventral surface. Lip brown. Maxillæ yellowish-brown with yellow tip. Sternum and legs yellow. Palp yellow except terminal joint which is brown. Abdomen below yellow with a median and two lateral longitudinal black bands. The median band is in reality composed of disconnected spots, begins about halfway

between genital fold and petiolus and extends backward almost to spinnerets. The two lateral bands begin immediately behind lungs and extend to spinnerets. Together, the two lateral bands would form a perfect letter U but for the lack of black color in front of the spinnerets. The spinnerets practically of the same color as the abdomen. Habitat: Cerro Flores.

Lycosa sanlorenziana sp. nov. (Figure 99).

Female. Total with chelicerae 12.5 mm. Carapace 5.270 mm. long, 3.740 mm. wide. Thoracic groove longitudinal. Head with slanting sides. First row of eyes shorter than second in ratio 36:46 and curved downward. Ratio of eyes AME:ALE: PME:PLE = 9:7:19:16. AME separated from each other by 2/9 of their diameter, from ALE by 2.5/9 of their diameter, from PME by 4/9 of their diameter. PME separated from each other by 11/19 of their diameter, from PLE by their diameter. PLE separated from each other by twice their diameter. Quadrangle of posteriors wider than long in ratio 66:48. Clypeus in front of AME equal to their diameter. A transverse row of four bristles on clypeus. A median very long black bristle between AME and a pair of very short black bristles immediately behind (and between) AME. A circular fringe of short white hair around PME, and around this fringe a wider circle of long black bristles. PLE surrounded by an imperfect circle of black long bristles. A median row of shorter black bristles extends from eyegroup to thoracic groove and ends in a pair. A row of black bristles on each side of the eyegroup extends from cephalothoracic sulcus to margin of carapace. Median and lateral bands of carapace clothed with white hair, the dark bands with black hair, the edge of the carapace except behind, with a narrow line of white hair. Clypeus receding so that the anterior eyes protrude beyond the edge of the carapace. *Chelicerae* with prominent yellow boss. Promargin with three teeth of which the median is strongest, the distal exceedingly small. Retromargin with three equal teeth. Scopula on promargin only. A brush of soft hair behind teeth of retromargin. *Lip* as wide as long, articulated to maxillae one-third from base. Anterior edge of lip slightly emarginate, fringed with bristles. *Maxillae* with brown scopula and black serrula. *Sternum* longer than wide in ratio 25:21, considerably narrowed in front, pointed behind. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	3.825	4.930	2.975	1.870	13.600
II	3.655	4.420	2.805	1.785	12.665
III	3.230	4.080	3.070	1.700	12.080
IV	4.420	5.610	5.185	2.210	17.425

First and second tarsi and metatarsi scopulate to base, scopulæ entire. Third and fourth tarsi scopulate to base, but scopulæ bisected longitudinally by a band of setæ. Third metatarsi scopulate in distal half, fourth metatarsi only at end. Trichobothria in two rows on all tibiæ and tarsi, in one row on all metatarsi. A long fine bristle on dorsal surface of all tarsi one-third from base. Upper claws on first and second pair of tarsi with 4 teeth, on third and fourth pair with 7 teeth. Third claw smooth. Palpal claw with 3 teeth.

Spines. First leg. Femur—dorsal 1-1-1, distal one small; prolateral 0-0-2; retrolateral 1-1-1, all small; ventral 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 1-1 fine bristles, prolateral 1-1, the first one quite small; retrolateral 0, ventral 2-2-2, the distal pair small. Metatarsus—ventral 2-2-2, distal pair small, elsewhere 0. *Second leg* same as first, except: Femur—prolateral 0-1-1. Metatarsus—ventral 2-2-3 (a median apical small spine in addition to the regular pair). *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 fine bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-3. *Fourth leg* same as third, except: Femur—retrolateral 0-0-1. *Palp.* Femur—dorsal 1-1-2, prolateral 1 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1, prolateral 1-1, elsewhere 0. Terminal joint—prolateral 2-1, retrolateral 1, elsewhere 0. Colulus small, but distinct. Epigynum of the usual type, shown in Figure 99, central bar widest and angular somewhat beyond middle, hence converging considerably anteriorly. Transverse arm rather short and almost straight. Color in alcohol: Carapace dark brown with a median and a pair of lateral longitudinal yellow bands. Median band begins between PME and is almost of the typical Greek letter Φ type, extending posteriorly to edge of carapace. Lateral bands submarginal, beginning to the outside of ALE and extending to posterior edge of carapace. Between the lateral band and the edge of the carapace on each side a narrow black band.

Marginal line white. Clypeus yellow with a longitudinal black band on each side extending from ALE to edge. Legs brown mottled with darker brown. Abdomen above greyish-brown with quite indistinct markings. Chelicerae in front dark at base, red-brown toward fang, red below. Lip, maxillæ, sternum and coxæ yellow. Legs below darker yellow mottled with brown. Abdomen below greyish-yellow, with four narrow, not well-defined longitudinal black lines extending from genital fold to spinnerets toward which they gradually converge. Spinnerets yellow, anterior pair with outer sides black. Habitat: Wilcox camp on San Lorenzo River.

Lycosa longitarsis F. Cambridge (Figure 100).

Female. Total with chelicerae 16.0 mm. Carapace 8.5 mm. long, 6.12 mm. wide. Thoracic groove longitudinal. Posterior declivity of carapace, as in most species of the genus *Lycosa,* forms with the base of the carapace an angle of about 45°. Head with slanting sides. First row of eyes shorter than second and slightly curved downward. Ratio of eyes AME:ALE:PME:PLE = 12:10:28:22. AME separated from each other by 4/12 of their diameter, from ALE by 5/12 of their diameter, from PME by 7/12 of their diameter. PME separated from each other by 16/28 of their diameter, from PLE by 24/28 of their diameter. PLE separated from each other by 50/22 of their diameter. Quadrangle of posteriors wider than long in ratio 80:70. Clypeus equal to the diameter of AME. A transverse row of 4 bristles on clypeus. PME surrounded by a circle of short, stout white hairs which have the appearance of rods with blunt end. Outside of the circle are long black bristles. Inner edge of ALE also fringed with white rodlike hairs. One black bristle between AME in line with their lower edge and a pair of black bristles a little above AME. An irregular circle of black bristles around each PLE and a middorsal row of black bristles, between eyegroup and thoracic groove, ending with a pair. On each side a row of black bristles extending from the level of ALE to cephalothoracic sulcus. The dark bands of the carapace clothed with short black hair, the longitudinal light bands and the marginal line with short white hair. *Chelicerœ* clothed with black bristles and long white hair. Boss well developed, but not conspicuous. Promargin with 3 teeth of which the median is largest, the distal smallest. Retro-

margin with 3 equal teeth. Scopula on promargin only, of yellow color. A soft brush of brown hair behind teeth of retromargin extending to base of cheliceræ. *Lip* a little longer than wide in ratio 13 : 12. *Maxillæ* wider in front than at base, with brown scopula and a comparatively short serrula. *Sternum* longer than wide in ratio 41 : 32, pointed behind, truncated in front, clothed with black bristles. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	6.545	8.160	4.930	3.060	22.695
II	6.120	7.820	4.420	2.805	21.165
III	5.440	6.885	5.100	2.720	20.145
IV	7.480	9.180	8.670	3.655	28.985

First and second tarsi and metatarsi scopulate to base, their scopulæ entire. Third and fourth tarsi scopulate to base, but their scopulæ bisected longitudinally by a broad band of setæ. Third metatarsus scopulate almost to base, its scopula bisected. Fourth metatarsus scopulate in anterior two-thirds, its scopula also bisected by setæ. Upper claws on first and second tarsi with 5 teeth, on third and fourth tarsi with 8 teeth. Third claw smooth. Palpal claw with 5 teeth. Trichobothria in two rows on all tibiæ and tarsi, in one row on all metatarsi. At end of basal third of all tarsi a long, fine, middorsal bristle. First and second dorsal femoral spines very long.

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 fine long bristles, elsewhere 0. Tibia—dorsal 0, prolateral 1-1, retrolateral 1 at end, very small, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1 at end, retrolateral 1 at end, ventral 2-2-1. *Second leg* same as first, except: Femur—prolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 fine bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-3. *Fourth leg* same as third, except: Femur—prolateral 1-0-1, retrolateral 1 at end. *Palp.* Femur—dorsal 1-1-2, prolateral 1 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1, prolateral 1-1, retrolateral 0, ventral 0. Terminal joint—prolateral 2-1, retrolateral 1, elsewhere 0. Epigynum shown in Figure 100, longitudinal bar wider in middle than at ends. Transverse bar distinctly procurved.

Color in alcohol: Carapace reddish-brown with a median and a pair of lateral longitudinal yellow bands. Median band narrow, extending forward to second row, backwards to edge of carapace. A procurved U-shaped yellow mark gives the longitudinal band the appearance of the Greek letter Ψ. Lateral bands submarginal, extending from clypeus to posterior edge. Marginal line narrow, white. Abdomen brown above, with a pair of triangular black spots in front and a white angular line formed by white hair, reaching beyond middle. Legs reddish-brown above, clothed with black and white hair. Cheliceræ dark reddish-brown. Lip and maxillæ reddish-brown with yellow tip. Sternum almost orange with yellow longitudinal median band with gradually fading sides. Coxæ and trochanters orange, legs yellowish-brown below. Abdomen light yellow below with a pair of rows of brown dots, bordered by brown. Spinnerets brown. Colulus well-developed. The species is closely allied to *L. dilatata.* Habitat: Panama, Costa Rica, Guatemala and Mexico.

Lycosa dilatata F. Cambridge (Figures 101-104).

A very variable species both as to size and coloration. The smallest mature females measure only 13 mm. in length, the largest fully 22 mm. In the type inadequately described by F. Cambridge, the venter of the abdomen has three longitudinal stripes. In some specimens it is entirely black, in others the color is altogether light. The epigynum, too, is subject to variation. Usually the longitudinal bar widens toward the end, but there may be in some cases a constriction near the end. The ratio of the length to the width is also not a reliable character.

a) Female from Bocas del Monte—Typical form.

Total with cheliceræ 16.0 mm. Carapace 8.0 mm. long, 6.0 mm. wide. Thoracic groove longitudinal. Head with slanting sides. First row of eyes shorter than second, slightly curved downward. Ratio of eyes AME:ALE:PME:PLE = 10:7:24:17. AME separated from each other by 3/10 of their diameter, from ALE by the same distance, from PME by 4/10 of their diameter. PME separated from each other by their radius, from PLE by 17/24 of their diameter. PLE separated from each other by 14/17 of their diameter. Quadrangle of posteriors wider than long in ratio 66:44. Promargin of *chelicerœ* with three teeth of

which the median is largest, the distal smallest. Retromargin with three fairly equal teeth. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	6.0	7.0	4.4	3.0	20.4
II	5.0	6.2	4.2	2.6	18.0
III	4.8	5.4	4.2	2.6	17.0
IV	6.8	7.7	7.2	3.3	25.0

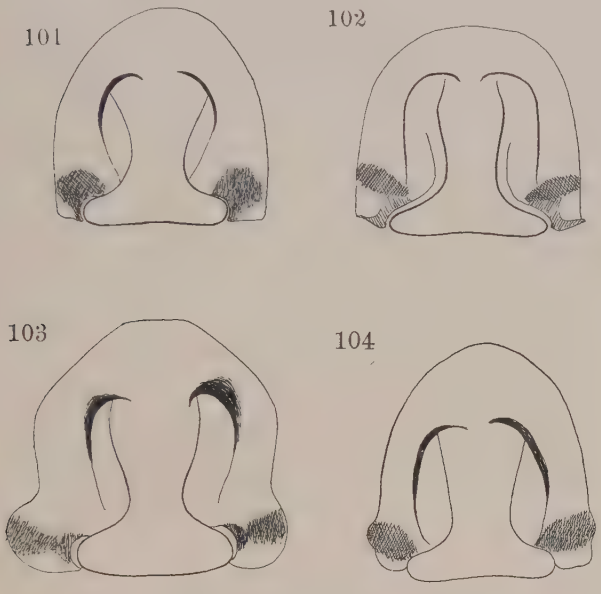


FIGURE 101, *Lycosa dilatata* F. Cambridge, epigynum of a specimen from Cerro Flores. FIGURE 102, Same, epigynum of a specimen from Bocas del Monte. FIGURE 103, Same, epigynum of a specimen from Culebra. FIGURE 104, Same, epigynum of a specimen from Cerro Flores.

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1-1-1 or 0-1-1, first spine very small on left leg, absent on right leg; retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 0, prolateral 1-1, retrolateral 0, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1 at end, retrolateral 1 at end, ventral 2-2-1. *Second leg* same as first, except: Femur—prolateral 1-1. Metatarsus—prolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 fine bristles, prolateral 1, retrolateral 1, ven-

tral 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-3. *Fourth leg* same as third, except: Femur—prolateral 1-0-1, retrolateral 0-0-1. Trichobothria in two rows on all tibiæ and tarsi, in one row on all metatarsi. First and second tarsi and metatarsi scopulate to base, their scopula entire. Third and fourth tarsi scopulate to base but their scopulæ bisected longitudinally by a broad band of setæ. Third metatarsi scopulate in distal half. A long, thin, middorsal bristle one-third from base on all tarsi. Epigynum shown in Figure 102. Color in alcohol: Carapace brown with a median, longitudinal light band extending from the eyes of the second row to posterior edge. It is of the typical Greek letter Φ type, clothed with white hair. Dark portions of carapace clothed with black hair. Edge of carapace with a very fine black line and a fringe of white hair. Abdomen above with a pair of triangular dark spots in front separated by a yellow ∧-shaped mark. Abdomen behind with indistinct, angular, transverse lines. Legs brown above, femora with darker spots. *Chelicerœ* black. Lip and maxillæ dark brown with yellow tip. Coxæ brown. Sternum brown with median yellow stripe in anterior two thirds. Abdomen below yellowish with three parallel, longitudinal black bands extending from genital fold to spinnerets, separated from each other by yellow and connected in front of spinnerets by black. Spinnerets brown. Legs brown below.

b) Female from Cerro Flores—black variety.

Total with chelicerœ 18.5 mm. Carapace 8.7 mm. long, 6.3 mm. wide. First leg 21.5 mm., fourth—26.0 mm. Spines as in the typical form. Epigynum shown in figure 101. Coloration same as in the typical form, but the ventral surface of the abdomen entirely black from the genital fold to the spinnerets.

c) Female from Culebra—light variety.

Total with chelicerœ 21.7 mm. Carapace 8.925 mm. long, 7.650 mm. wide. Thoracic groove longitudinal. Head with slanting sides. First row of eyes shorter than second in ratio 54:68, slightly curved downward. Ratio of eyes AME:ALE: PME:PLE = 13:9:30:24. AME separated from each other by 4/13 of their diameter, from ALE by the same distance, from PME by 6/13 of their diameter. PME separated from each other by their radius, from PLE by 23/30 of their diameter.

PLE separated from each other by two diameters. Quadrangle of posteriors wider behind than in front in ratio 84:66, wider than long in ratio 84:66. Clypeus equal to the diameter of AME. Posterior four eyes fringed with a semicircle of white hair and a few black bristles. A row of black bristles on each side extends from ALE to cephalothoracic sulcus. Median longitudinal band clothed with short white hair, rest of carapace with short black hair. Marginal line black with some white hair and black bristles in intercoxal spaces. Posterior margin devoid of hair. Posterior declivity 45°. Clypeus receding, so that eyes of first row protrude beyond edge of carapace. *Cheliceræ* with strong red boss. Promargin with three teeth of which median is largest, distal smallest. Retromargin with three equal teeth. Scopula on promargin only. A brush of soft hair behind teeth of retromargin extending to base of cheliceræ. *Lip* longer than wide in ratio 16:13, articulated in middle to maxillæ. Lateral excavations long. *Maxillæ* wider at end than at base, evenly curved on outside, so that viewed from below they appear to be pointed at the proximal end. Scopula well developed, brown. Serrula present. *Sternum* oval, longer than wide in ratio 40:33, pointed behind, clothed with erect black bristles and fine short black hair. Trochanters deeply notched. First and second tarsi and metatarsi scopulate to base, their scopulæ entire. Third and fourth tarsi scopulate to base, but their scopulæ bisected longitudinally by a broad band of setæ. Their metatarsi slightly scopulate in distal third. *Legs* 4123.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	6.205	7.905	4.675	2.805	21.590
II	5.610	7.480	4.505	2.550	20.145
III	5.270	6.430	4.505	2.550	18.755
IV	6.970	8.500	7.820	2.975	26.265

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1 fine bristles, elsewhere 0. Tibia—dorsal 0, prolateral 1-1, retrolateral 0-1 on right leg, 1-0 on left leg, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1 at end, retrolateral 1 at end, ventral 2-2-1. *Second leg* same as first, except: Femur—prolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 1-1-1, ventral 0. Patella—dorsal 1-1, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2.

Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2-3. *Fourth leg* same as third, except: Femur—retrolateral 0-0-1 and on the right leg dorsal 1-1-1-1. A long, fine middorsal bristle one third from base on all tarsi. Trichobothria in two rows on all tibiæ and tarsi, in one row on metatarsi. Upper claws on first and second tarsi with from 4 to 5 teeth, on third and fourth tarsi with from 8 to 9 teeth. Third claw smooth. Palpal claw with 4 teeth. Epigynum as shown in Figure 103. Color in alcohol: Carapace light brown with a median longitudinal yellow band of the typical Greek letter Φ shape. Cheliceræ black. Legs light brown. Abdomen above greyish-yellow with two triangular black spots in front, which are formed by long black hairs. A light ∧-shaped mark as in the typical form, but no transverse lines behind it. Below, cheliceræ dark brown, lip and maxillæ dark brown with yellow tip, sternum brown with a median yellow stripe in anterior three quarters. Coxæ and trochanters yellowish-brown, first and second femora yellow, third and fourth femora slightly darker, rest of all legs yellowish-brown. Abdomen below buff with a black median longitudinal band extending from epigynum almost to spinnerets and with a pair of lateral bands which extend from genital fold to spinnerets where they unite, but which are not as heavy as median band and are broken up in places. Spinnerets yellowish-brown, anterior pair as well as posterior clearly two-jointed.

From the foregoing descriptions it is evident that the most constant characters of this species are the yellow median longitudinal band on the carapace, the yellow median stripe on the sternum, the position of the prolateral spines on the first femora, the middorsal bristle on the tarsi and last, but not least, the proportion of the legs and eyes. Habitat: Panama, Mexico.

Family Senoculidæ

Senoculus valentinei sp. nov. (Figures 105-111).

Of the two males of this interesting species, collected by Mr. Valentine, one is 13.3 mm. long, the other 15.3 mm. and has the appearance of a much larger and heavier spider. Nevertheless both specimens agree closely in structure and coloration. The smaller specimen being better preserved, was chosen for detailed description.

Male. Total without cheliceræ 13.3 mm. Carapace 4.335 mm. long, 3.400 mm. wide in middle, 1.530 mm. wide at anterior margin. Eyegroup 1.785 mm. wide. Abdomen 8.075 mm. long to end of anal tubercle, 2.295 mm. wide, cylindrical.

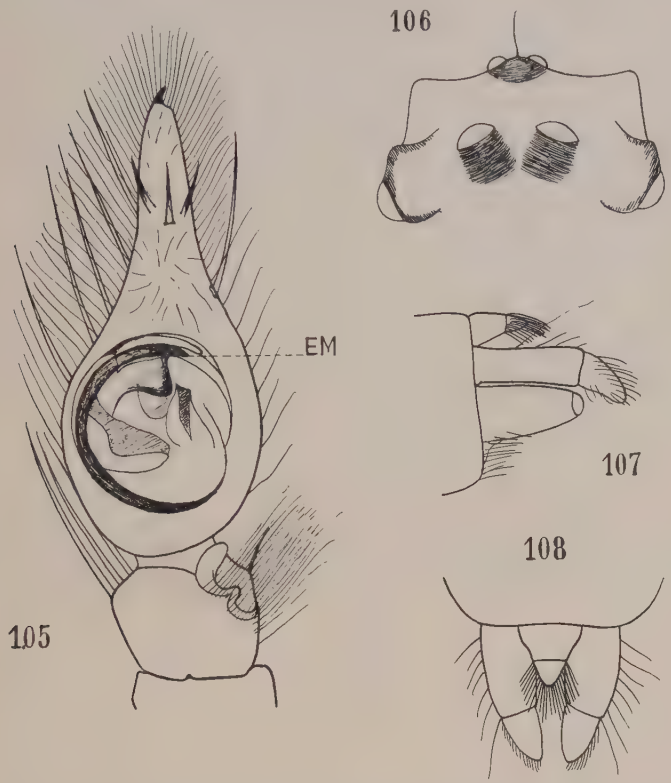


FIGURE 105, **Senoculus valentinei** sp. nov., left palp from below. FIGURE 106, Same, eyegroup from above, anterior lateral eyes not visible. FIGURE 107, Same, spinnerets in side view. FIGURE 108, Same, spinnerets and anal tubercle viewed from above.

Carapace very flat, depressed in middle, almost round if portion carrying AME be disregarded. This portion has parallel sides. Thoracic groove longitudinal, but so faint that its presence may be ascertained only by the distribution of the hair on the carapace and the position of the radiating thoracic sulci. The carapace is clothed with recumbent, fine, white hair and recumbent, fine, brown hair. Erect, dark hair on margins. *Eyegroup* characteris-

tic of the genus. Eyes eight in two rows, but the first row so strongly recurved that the ALE may be regarded as belonging to the posterior row, the AME being far in front of the others, almost on the edge of the carapace. Posterior row also recurved, but not nearly as much. ALE are oval, ratio between long and short axis being 4 : 3. They are nocturnal, opalescent white, smaller than all the other eyes, which are round and diurnal. Ratio of eyes AME : ALE : PME : PLE = 5 : 4 : 8 : 8. AME separated from each other almost by their diameter, from ALE by five

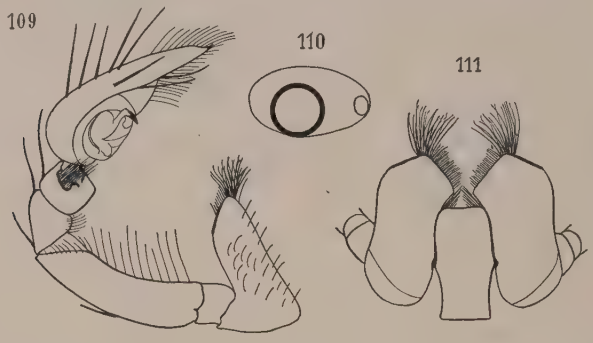


FIGURE 109, **Senoculus valentinei** sp. nov., right palp from outside. FIGURE 110, Same, right lateral eyetubercle showing posterior lateral eye and the much smaller, oval, anterior lateral eye. FIGURE 111, Same, lip and maxillæ.

diameters. PME are separated from each other by 7/8 of their diameter and from the PLE by slightly more than one and one half diameters. Laterals are on a prominent joint tubercle shown in Figure 110. Quadrangle much narrower in front than behind in ratio 12 : 24, as long as wide behind. Clypeus smaller than diameter of AME. AME are situated on a small tubercle and there is between them a single black bristle. *Chelicerœ* with a strong boss. Both margins with 3 teeth. On the promargin the distal tooth is smaller and somewhat in advance of the others. On the retromargin the teeth are equal and equidistant. A scant scopula on the promargin only. *Lip* free, much longer than wide in ratio 2 : 1, narrowed toward base, articulated in middle to maxillæ one-third from their base (Figure 111). Lip sparsely clothed with short dark hair. Anterior edge with black bristles inclined medially. *Maxillæ* long, almost parallel, outer edge slightly

concave. Scopula and serrula shown in Figure 111. *Sternum* almost in the shape of a rhombus, anteriorly truncated and but slightly wider than lip, posteriorly pointed, widest between second and third coxæ, longer than wide in ratio 60:45, sparsely clothed with hair of varying length. Coxæ clothed with white hair. A long, black bristle in middle of each coxæ. Trochanters all notched and with the usual fringe of hair in middle. Legs with numerous spines and with a brush of long hair below on all tibiæ, metatarsi and tarsi. Dorsally legs are clothed with long, erect hair and short, black, recumbent hair; also with plumose hair which is fairly numerous, though difficult to see. The spines are long. Upper claws similar on all tarsi, with a row of from 6 to 7 teeth the distal three of which are the longest. Third claw with three teeth. The claws are situated on a distinct onychium which is of the same thickness as the tarsus, but clearly separated from the latter by a narrow band devoid of hair. There are no true serrated bristles on the tarsi. Trichobothria are apparently present only on the tarsi where one can see three pairs of them. But even these are small, and it is possible that trichobothria escape observation on account of the comparatively long hair. *Legs* 1243.

	Femur	Pat. + Tibia	Metatarsus	Tarsus	Total
I	6.120	8.755	6.290	1.955	23.120
II	5.100	8.075	4.930	1.700	19.805
III	3.740	4.165	3.400	1.275	12.580
IV	5.440	5.440	4.675	1.700	17.255

Spines. First leg. Femur—dorsal 1-1-1-1, prolateral 1-1-1, retrolateral 1-1-1-1, ventral 0. Patella—dorsal 1-1, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 1, retrolateral 1, ventral 2-2-2-2. Metatarsus—dorsal 0, prolateral 1-1-1, retrolateral 1-1-1-1, ventral 2-2-2. *Second leg* same as first, except: Femur—dorsal 1-1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1-1, retrolateral 1, ventral 0. Patella—dorsal 1-1, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, prolateral 1-1, retrolateral 1-1, ventral 2-2-2. Metatarsus—dorsal 1-1-1, prolateral 1-1-1, retrolateral 1-1-1, ventral 1-1-2. *Fourth leg* same as third, except: Femur—dorsal 1-1-1-1, prolateral 1-1-1-1, retrolateral 1-1-1. Tibia—ventral 2-2. *Palp.* Femur—dorsal 1-1, elsewhere 0. Patella—dorsal 1-1, prolateral 1, elsewhere 0. Tibia—dorsal 1, prolateral 4 in a bunch, elsewhere 0. Terminal

joint—2-2 ventral ones on cymbium in front of alveolus and numerous dorsal spines. Femur of palp distinctly curved downward. Tibia globular, when viewed from the inside. When viewed from the outside it discloses the presence of a pouch with hair (Figure 109). There is no claw at end of cymbium, but the cymbium itself terminates in a sharp point. *Abdomen* long, cylindrical. Anal tubercle unusually long, two-jointed, cone-shaped (Figure 108). Spinnerets six. Anterior pair heavier than posterior pair, two-jointed, but terminal joint quite short, hemispherical. Anterior spinnerets contiguous. Colulus wanting. Posterior spinnerets two-jointed and much longer than the anterior pair. Terminal joint cylindrical, shorter than basal joint. Color in alcohol: Below, more or less uniformly yellow with cheliceræ darker. Above, generally yellow with blackish spots. Edge of carapace dark. Cymbium dark. Dark spots on femora and patellæ and at both ends of tibiæ. End of anal tubercle and terminal joint of posterior spinnerets also dark. Abdomen above distinctly purplish with heart clearly visible. Behind the heart a white median mark with two pairs of oblique dark spots converging to middorsal line anteriorly. Anterior end of abdomen with a dark median space and dark lateral angles. Posterior third of abdomen with two indistinct dark spots confluent near anal tubercle. Habitat: Wilcox camp on San Lorenzo River.

Family Attidæ

Amycus palpinalis F. Cambridge (Figures 112-117).

Male. Total with cheliceræ 5.76 mm. Carapace 2.542 mm. long, 2.050 mm. wide, 1.804 mm. high. Ratio between length of eyegroup and posterior declivity 42:44. Chitin of eyeregion orange and clearly deliminated from the pale yellow of the rest of the carapace. Eyeregion emarginate posteriorly by the length of the thoracic groove which is longitudinal and is situated immediately behind the eyeregion. AME on a cylindrical, orange base. ALE and PME on each side in a joint black spot. ALE prominent. PLE elevated, on a black spot. First row of eyes strongly recurved, wider than second row in ratio 43:35 and slightly wider than third row in ratio 43:41. Eyegroup as long as wide posteriorly. Ratio of eyes AME:ALE:PME:PLE = 18.5:8:2:8. AME separated from each other by about 1/20 of their diameter and from ALE by the same distance. Eyes of second row

(PME) separated from ALE by 7.5/8 of the diameter of the latter, from PLE by 9.5/8 of the diameter of the latter, *i. e.* eyes of second row are situated slightly nearer ALE than PLE. Clypeus 21/18.5 of the diameter of AME, dark, without scales or hair. Head depressed between the eyes, glabrous, with a fringe of fine, red hair around each AME, small tufts of fiery red hair to the inside of ALE and also between PME and PLE on each side. A transverse row of 4 bristles behind each AME, two bristles to

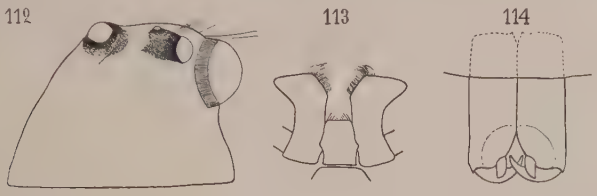


FIGURE 112, *Amycus palpinalis* F. Cambridge, side view of carapace. FIGURE 113, Same, lip and maxillæ. FIGURE 114, Same, cheliceræ of male, front view.

the outside of ALE and one bristle to the outside of PLE. *Cheliceræ* withdrawn deeply into the cephalothorax, with parallel edges and parallel to each other, visible portion barely longer than clypeus. Basal joint excavated on prolateral surface. Margins oblique. Promargin with a strong, flat, wide tooth of peculiar shape, inasmuch as it is with a straight dorsal and a curved ventral edge. There is also a sharp, triangular tooth near the distal end of promargin, but belonging to the prolateral surface just outside the articulation of the fang. Retromargin with 6 small teeth. Scopula on promargin only and very thin. Boss wanting. Fang comparatively short and stout. Its dorsal surface evenly rounded, but the ventral one bent in two places thus forming a straight minutely serrated edge in its median portion. *Lip* longer than wide in ratio 14:11, articulated to maxillæ in middle. *Maxillæ* long, parallel. Outer angle strongly produced and this projection chitinized. Anterior edge straight, truncature short, with a brown scopula. Serrula dorsal to anterior edge, extending from end of scopula almost to outer angle. *Sternum* oval, widest between second and third coxæ, truncated anteriorly, longer than wide in ratio 29:23. First coxæ separated by more than width of lip, fourth coxæ sub-contiguous. Edge of sternum chitinized. *Legs* 3142.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.845	1.066	1.558	1.107	0.615	6.191
II	1.722	0.902	1.312	1.066	0.574	5.576
III	2.460	0.943	1.476	1.435	0.656	6.970
IV	1.968	0.656	1.230	1.435	0.615	5.904
Palp	1.435	1.230	0.820	0.635		4.110

Spines. First leg. Femur—dorsal 1-1-1 (1-1-1-1 on left leg), prolateral 2 at end, elsewhere 0. Patella—0. Tibia—dorsal 1-1-1-1-1 bristles of which the first and the last are erect; prolateral 1-1-1, retrolateral 0, ventral 2-2-2 long spines. Metatarsus —ventral 2-2 long spines, elsewhere 0. *Second leg* same as first.

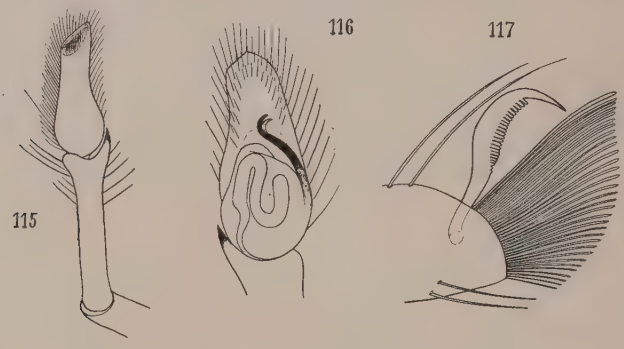


FIGURE 115, *Amycus palpinalis* F. Cambridge, right palp from above. FIGURE 116, Same, right palp from below. FIGURE 117, Same, end of fourth tarsus.

except: Femur retrolateral 1 at end. Patella—dorsal 0-1 bristle. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-2, elsewhere 0. Patella—dorsal 0-1 bristle, retrolateral 1, elsewhere 0. Tibia—dorsal a row of weak bristles, prolateral 1-1-1, retrolateral 1-1-1, ventral 1p-1r. Metatarsus—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2. *Fourth leg* same as third. No spines on palp. Trichobothria in two rows on tibiæ, in one row on metatarsi and tarsi. Claws 2, with a long series of teeth. Trochanter of *palp* three times longer than wide. Tibial apophysis simple. Cymbium obliquely truncated at end. Embolus originating from middle of bulb on the inside. End of embolus forked. *Abdomen* cylindrical. Genital fold straight. Colulus well developed. All six spinnerets cylindrical, dark brown. Anterior pair heaviest, but all spinnerets of the same length. Anterior spinnerets contiguous,

posterior pair separated from each other by the median pair. Legs and abdomen sparsely clothed with simple hair. Scales wanting. Dorsum with dark spot in front, followed by a pair of longitudinal dark bands. Legs light yellow as far as end of femora, brown from here on. Habitat: Wilcox camp on San Lorenzo River.

Amycus spiralifer F. Cambridge (Figures 118 and 119).

Male. Total 4.100 mm. Carapace 1.517 mm. long, 1.184 mm. wide, 0.999 mm. high. The carapace is as wide as the first row of eyes, but slightly wider than posterior row which measures 1.073 mm. Eyegroup 0.999 mm. long, posterior declivity from PLE to edge of carapace 0.851 mm., *i. e.* the eyegroup occupies more than half of the carapace. Head flat. Eyes prominent. Thoracic groove longitudinal. Area between eyes yellowish-brown, rest of carapace almost black. Clypeus thickly clothed with converging white hair. A tuft of white hair behind the point at which AME touch each other. Another tuft to the inside of each ALE, and still another on each side between PME and PLE. Bristles small and fine. Ratio of eyes AME : ALE : PME : PLE = 12 : 5 : 1.25 : 5. Clypeus equal to 7/12 of the diameter of AME. AME contiguous, elevated on a cylindrical base, contiguous with ALE. PME situated nearer ALE than PLE in ratio 5 : 7.5. First row of eyes 1.184 mm. wide, second row 0.962 mm., third row 1.073 mm. Viewed from in front, first row is curved upward by centers, but slightly downward by upper edges. *Chelicerœ* brown, short and weak, with parallel sides. Basal joint with a strong middorsal tooth in middle, directed forward. Promargin with a single tooth, retromargin with 4 teeth. Scopula on promargin only and rather thin. *Lip* as long as wide, brown with yellow tip. *Maxillæ* light brown, with long truncature. Outer angle produced, but not nearly as much as in *A. palpinalis*. *Sternum*, shield-like, widely truncated in front, pointed behind. Fourth coxæ sub-contiguous. Anteriorly sternum wider than lip. First leg dark brown, lighter toward end. Second, third and fourth leg and sternum light yellow. *Legs* 1432.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.221	0.666	1.036	0.777	0.444	4.144
II	0.999	0.518	0.666	0.592	0.407	3.182
III	1.332	0.518	0.777	0.740	0.444	3.807
IV	1.295	0.407	0.814	0.925	0.407	3.848
Palp	0.555	0.222	0.222	0.444		1.443

Patella of *palp* 0.148 mm. wide. Trochanter short, 0.148 mm. long and 0.130 mm. wide. Tibia with a lateral blunt apophysis and an anterior pointed apophysis. Embolus wound up spirally.

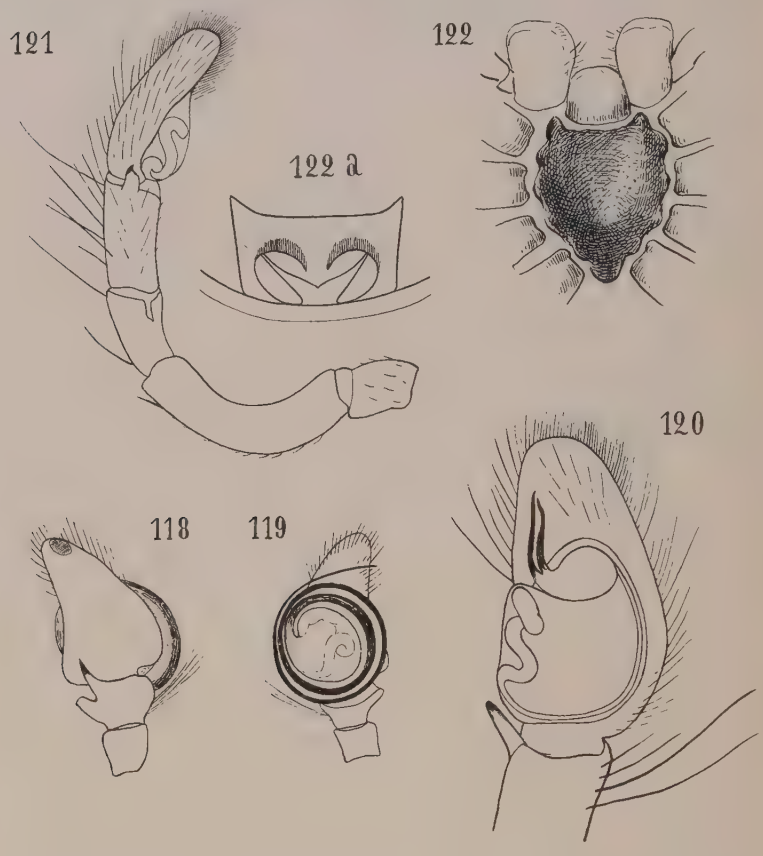


FIGURE 118, *Amycus spiralifer* F. Cambridge, left palp from above. FIGURE 119, Same, left palp from below. FIGURE 120, *Cobanus subfuscus* F. Cambridge, right palp from below. FIGURE 121, Same, right palp in side view. FIGURE 122, **Cylistella castanea** sp. nov., sternum, lip and maxillæ. FIGURE 122-a, Same, epigynum.

Spines transparent, weak, in some places difficult to see. *First leg.* Femur—dorsal 1-1-1, prolateral 1-2, elsewhere 0. Patella—dorsal 0-1 bristle. Tibia—dorsal 0, prolateral 1-1-1, retrolateral 0, ventral 2-2-2, second pair nearer to the third than to the first pair. Metatarsus—dorsal 0, prolateral 1 at end, retrolateral 1 at end,

ventral 2-2. *Second leg* same as first, except: Femur—prolateral 0-1, retrolateral 0-1. Patella—0. Tibia—prolateral 0-1-1, ventral 1r-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 0-1, retrolateral 0-1, ventral 0. Patella—retrolateral 1, elsewhere 0. Tibia—dorsal 0, prolateral 0-1, retrolateral 0-1, ventral 1p-1r. Metatarsus—dorsal 0, prolateral 1-1, retrolateral 1-2, ventral 2-2. *Fourth leg* same as third, except: Patella—0. Tibia—prolateral 0, retrolateral 1-1-1, ventral 0. Metatarsus—retrolateral 1-1, ventral 0-2. No spines on palp. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Claws with a long, even row of teeth. Abdomen widest in anterior third, a little behind genital fold. Spinnerets cylindrical, all six equally long, but anterior pair heaviest. In place of a colulus a few hairs. Habitat: Guatemala and Panama.

Cobanus subfuscus F. Cambridge (Figures 120 and 121).

Male. Total with cheliceræ 5.376 mm. Carapace 2.542 mm. long, 1.968 mm. wide, 1.517 mm. high. First row of eyes 1.845 mm. wide, second row 1.681 mm., third row 1.763 mm. Viewed from in front, first row is straight by upper edges, curved upward by centers. Clypeus low, 3.5/15 of the diameter of AME. Ratio of eyes AME:ALE:PME:PLE = 15:9.5:2:8.5. Eyearea 1.369 mm. long, posterior declivity 1.591 mm. long. Eyearea wider than long. AME and ALE elevated on cylindrical base. PME nearer PLE than ALE in ratio 7:12, or if distance between PME and ALE be measured only to base of collar, in ratio 7:8. AME contiguous and sub-contiguous with ALE. Thoracic groove longitudinal, well developed. The black surrounding ALE with PME is confluent with the black surrounding PLE. White lanceolate hair between all eyes. Bristles rather weak, but regular in their distribution. Three bristles in a triangle on clypeus, with apex directed posteriorly. Two bristles on each side in front of AME. Three bristles in a triangle between AME, with apex directed anteriorly. One bristle on each side to the outside of ALE, one between ALE and PME, one to the outside of PME and one to the outside of PLE. *Cheliceræ* of peculiar form, inasmuch as their outer edges are strongly divergent, while at the same time their inner edges are parallel to each other. Promargin with 2 small, sharp teeth, situated close to proximal end. Retromargin with 2 strong, sharp teeth situated close to distal end.

Fang long, sinuous. There is no true scopula, but some hair on promargin and a row of hairs on retromargin. *Lip* longer than wide at base in ratio 12:10, rounded in front, articulated to maxillæ in middle, with sides slightly converging. Lip reaches beyond middle of maxillæ. *Maxillæ* slightly converging. Truncature short, thickly clothed with a brown scopula, the truncatures strongly diverging. Outer angle produced strongly, but rounded at end. Serrula unusually long, following the curvature of the outer angle and continuing along the outer edge close to the insertion of the trochanter. *Sternum* longer than wide in ratio 29:21, somewhat narrower in front than behind, truncated anteriorly to the width of the lip, bluntly pointed behind in front of hind coxæ which are contiguous. First pair of legs heavier than the others, with a fringe of stiff hair in the following places: along retrolateral edge of femur, prolateral and ventral on patella and tibia, ventral on metatarsus. This latter fringe is dark grey with a yellow tinge, the others brilliant yellow. A dark grey middorsal fringe is also present on the femur. Second femur has a middorsal and retroventral fringe of light yellow color. Third femur has a retroventral fringe of still lighter color. Trichobothria in two rows on all tibiæ, in one row on all metatarsi and tarsi. Claws with a long series of teeth. First femur flat below, but first tibia and metatarsus rounded. Femur of palp very strongly curved downward, forming an arc greater than 1/4 of a circle. Trochanter of palp moderately long, less than twice as long as wide. Tibia of palp with a straight, long apical apophysis on retrolateral side. Embolus slightly curved, cleft almost to base, but its two rami can be seen only in a certain position, this being the reason why F. Cambridge has figured it not as a forked organ, but entire. *Legs* 1342.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.886	1.107	1.681	1.189	0.697	6.560
II	1.640	0.820	1.148	0.984	0.574	5.166
III	1.845	0.820	1.107	1.107	0.656	5.535
IV	1.722	0.697	1.148	1.271	0.615	5.453
Palp	0.777	0.407	0.444	0.666		2.294

Spines. First leg. Femur—dorsal 1-1-1p, prolateral 1 at end, elsewhere 0. Patella—dorsal 0-1 bristle, prolateral 1, elsewhere 0. Tibia—dorsal 0, prolateral 1-1-1v, retrolateral 0-1, ventral 2-2-2. Metatarsus—dorsal 0, prolateral 1, retrolateral 1, ventral

2-2-2. *Second leg* same as first, except: Femur—prolateral 2 at end. Patella—dorsal 1-1 bristles. Tibia—ventral 1r-2-2. Metatarsus—prolateral 1-1, retrolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1 small spine at base, prolateral 1-1-1, retrolateral 1-1-1, ventral 0-0-2. Metatarsus—dorsal 1r at base, prolateral 1-2, retrolateral 1-1, ventral 2-2. *Fourth leg* same as third, except: Femur—prolateral 0. Tibia—retrolateral 0-1, ventral 1p-0-2. Metatarsus—prolateral 1-1-2, retrolateral 1-1-1, ventral 1p-2. *Palp* has no spines, but only bristles distributed as follows: Femur—dorsal 0-1. Patella—dorsal 1-1. Tibia—dorsal 1r-1 and several retrolateral bristles of light color. The distal prolateral and retrolateral spines of the first tibia are so much out of alignment with the other spines that they have been described by F. Cambridge as belonging to the ventral surface. A comparison of the first tibia with the second discloses the true nature of these spines. Anal tubercle large, cone-shaped. Spinnerets 6, of equal length, cylindrical. Anterior pair heaviest, contiguous. Posterior pair separated by the width of second pair. Colulus small, but distinct. Tracheal spiracle wide, procurved, close to spinnerets. Abdomen sparsely clothed with simple hair. Color in alcohol: Carapace orange, with black around the eyes, forming a band on right and left side. First leg orange with yellow tarsus. Other legs yellow with a dark ring at end of metatarsi. Below, dark yellow. Abdomen above buff with a pair of dark, longitudinal bands extending to middle, followed by 5 transverse recurved arcs. Habitat: Panama, Costa Rica.

Cylistella castanea sp. nov. (Figures 122 and 122a).

Female. Total 1.88 mm. Carapace 0.814 mm. long, 0.888 mm. wide, posterior declivity vertical, beginning 0.259 mm. behind PLE. Abdomen 0.962 mm. long, 1.110 mm. wide. Eyes of third row protruding beyond the edge of the carapace. Surface of head convex, devoid of hair, covered with "needleprick" impressions. Viewed from in front, first row of eyes straight by centers, 0.814 mm. wide, second row 0.88 mm., third row 0.814 mm. Eye-group wider than long in ratio 24:15. Ratio of eyes AME:ALE:PME:PLE = 7:3.5:0.75:3. AME contiguous and sub-contiguous with ALE. PME situated in middle between ALE and

PLE. Clypeus 2/7 of the diameter of AME. *Chelicerœ* exceedingly weak, shorter than maxillæ. Promargin smooth, with a row of hairs. Retromargin with 3 teeth. *Lip* as wide as long, rounded in front. *Maxillœ* slightly converging, with long scopula and rounded outer angle. *Sternum* convex, longer than wide, produced between hind coxæ. First coxæ wide apart. *Legs* 4123.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	0.407	0.222	0.185	0.148	0.222	1.184
II	0.407	0.185	0.185	0.148	0.185	1.110
III	0.370	0.185	0.164	0.148	0.164	1.031
IV	0.481	0.222	0.222	0.164	0.202	1.291
Palp	0.148	0.111	0.127	0.164		0.550

Spines too difficult to examine without dismembering the specimen. Abdomen wider than long, with a chitinized shield above, truncated in front. This truncated surface also chitinized, but not nearly as much as the dorsum. It is of lighter color and is separated from the dorsal shield by a narrow line of soft chitin. Shield shiny. Entire abdomen clothed with short hair. Spinnerets elevated. Anterior pair much heavier than the others. Colulus represented by a transverse row of hairs. Tracheal spiracle immediately in front of spinnerets, in a chitinized area. Epigynum flat, in a chitinized trapeze-shaped field. Color in alcohol: Carapace and dorsal shield of abdomen dark chestnut brown, shiny. Sides and venter of abdomen soft, grey. Spinnerets grey. Legs from patella on yellow with a dark spot at base of tibiæ below. Rest of spider brown. Habitat: Wilcox camp on San Lorenzo River.

Corythalia sulphurea (F. Cambridge) (Figures 123-128).

This is a somewhat variable species. For this reason a female and two males are here described in detail. The male from Cerro Flores conforms fully to the description given by F. Cambridge and has the same coloration. The male from San Lorenzo undoubtedly belongs to the same species, though of slightly different proportions.

a) Male from Cerro Flores. Total 4.40 mm. Carapace 2.132 mm. long, 1.517 mm. wide, 1.036 mm. high. Thoracic groove longitudinal, its anterior end touching imaginary line tangent to posterior edges of PLE. Eyearea 0.962 mm. long, thoracic portion 1.184 mm. long. First row of eyes 1.406 mm.

wide, second row 1.258 mm., third row 1.332 mm. PME nearer PLE than ALE in ratio 2:3. Ratio of eyes AME:ALE:PME:PLE = 11.5:8:2:7. Clypeus 1/3 of the diameter of AME. First row of eyes, when viewed from in front, curved upward by upper edges. AME sub-contiguous, distinctly separated from

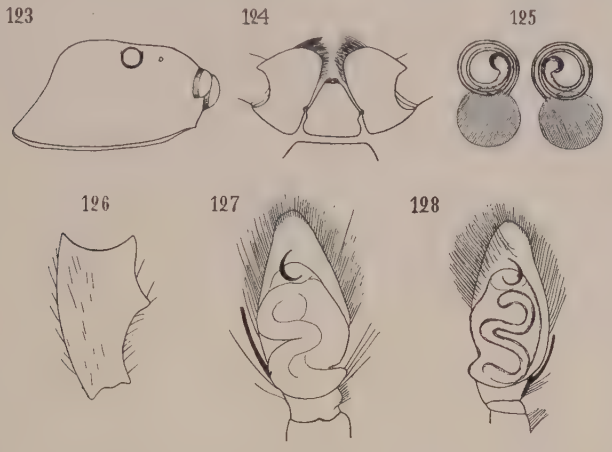


FIGURE 123, *Corythalia sulphurea* (F. Cambridge), carapace of male in side view. FIGURE 124, Same, lip and maxillæ of male. FIGURE 125, Same, epigynum. FIGURE 126, Same, right femur of male palp from below. FIGURE 127, Same, right palp of a male from Cerro Flores from below. FIGURE 128, Same, left palp of a male from San Lorenzo from below.

ALE. A row of black bristles fringing clypeus. Three bristles in a triangle on clypeus between AME and a pair of bristles on each side in front of ALE. Head and rear of carapace very dark brown, shiny, clothed with black hair. A lateral band of white scales on thoracic portion of carapace on each side reaching to margin. *Chelicerœ* without excavations. Promargin with flap fringed with 8 short, stiff hairs followed by 2 teeth proximally. Retromargin with a single sharp tooth. *Lip* more or less triangular with rounded end and rounded angles at base, as long as wide, surpassing middle of maxillæ. *Maxillæ* with rounded end. Outer angle sharply produced in the shape of a spur. Scopula dark grey. Serrula very short, straight, not quite reaching base of outer angle. *Sternum* convex, truncated in front to more than width of lip, a little longer than wide in

ratio 22:17. First coxæ almost twice as wide apart as width of lip. Fourth coxæ contiguous. Sternum, lip and maxillæ yellowish-brown, darker than coxæ, clothed with black hair. Legs clothed with black hair which is rather sparse. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts weak, grey. Claws dissimilar. On first and second tarsus proclaw with a long series of closely set teeth, retroclaw with but 4 teeth. On third and fourth tarsi proclaw similar to the one on anterior tarsi, retroclaw with 6 teeth making the dissimilarity less than in anterior tarsi. *Legs* 1432.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.332	0.703	0.925	0.814	0.444	4.218
II	1.073	0.592	0.592	0.629	0.370	3.256
III	1.221	0.592	0.740	0.888	0.370	3.811
IV	1.147	0.555	0.830	1.036	0.444	4.012
Palp	0.555	0.296	0.148	0.518		1.517

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1 at end, elsewhere 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1-1-1-1 bristles, prolateral 1 at end, retrolateral 0, ventral 2-2-2, first pair near base. Metatarsus—dorsal 0, prolateral 1 at end, retrolateral 0, ventral 2-2. *Second leg* same as first, except: Femur—retrolateral 1 at end. Tibia—prolateral 1-1, ventral 1r-1r-2. Metatarsus—prolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, retrolateral 1, elsewhere 0. Tibia—dorsal 0-1 bristle, prolateral 1-1, retrolateral 1-1, ventral 1p-2. Metatarsus—dorsal 0, prolateral 1-2, retrolateral 1-2, ventral 2-2. *Fourth leg* same as third, except: Tibia—prolateral 1-1-1, retrolateral 1-1-1. Metatarsus—prolateral 1-1-2, retrolateral 1-1-2. *Palp.* Femur 0. Patella—dorsal 1 bristle, prolateral 2 bristles, elsewhere 0. Femur, patella and tibia yellow, thickly clothed with white hair. Terminal joint dark brown. Second, third and fourth pair of femora dark brown. Transverse bands on abdomen with light yellow scales. Otherwise dorsum of abdomen of brown chitin mottled with yellow, devoid of hair or scales. Median and posterior spinnerets and the anal tubercle light yellow, anterior spinnerets brown with base and terminal joint light yellow. Anterior spinnerets much stouter than the others. Posterior spinnerets, separated from each

other by more than the width of the anal tubercle, forming a straight line with the median pair which are situated between the posterior spinnerets. Venter brown. Bulb of palp with a rather long protuberance at base and femur of palp with a very distinct protuberance on inside in middle.

b) Male from San Lorenzo. Total with spinnerets 3.485 mm. Carapace 1.776 mm. long, 1.480 mm. wide, 1.110 mm. high, widest in region of anterior eyes which are slightly projecting laterally. Head convex except between PLE where it is distinctly depressed. Thoracic groove longitudinal, its anterior end reaching just beyond imaginary line tangent to posterior edges of PLE. Eyearea 0.962 mm. long, posterior declivity 0.962 mm. long, very steep, beginning immediately behind eyes. First row of eyes 1.480 mm. wide, second row 1.258 mm., third row 1.295 mm. PME nearer PLE than ALE in ratio 4:7. Ratio of eyes AME:ALE:PME:PLE = 12:8.25:2:7. AME sub-contiguous and also sub-contiguous with ALE. Clypeus 3/12 of the diameter of AME. Viewed from in front, first row of eyes distinctly curved upward by upper edges. Three bristles in a triangle on clypeus between AME and one pair of bristles on each side in front of ALE. Edge of clypeus with a fringe of hair in a single row. AME in a circle of brown scales and a few brown scales to the inside of ALE. Head dark brown with short black hair and long black bristles irregularly arranged. A few white scales between ALE and PLE on each side to the inside of PME. A V-mark of white scales, with apex directed forward between PLE immediately in front of thoracic groove. The two ends of the mark and the posterior end of the thoracic groove are on the same level. A broad band of white scales on the sides of the thoracic portion of carapace, but the edge itself black. *Cheliceræ* without excavations, normal, brown with a few black hairs on anterior surface. Promargin with a flap fringed with 6-8 stiff, short dark hairs followed proximally by 2 small teeth. Retromargin with one sharp tooth. Fang short, slightly curved, stout at base. *Lip* very peculiar, triangular, much wider than long in ratio 9:6, considerably surpassing middle of maxillæ, squarely truncated at end, articulated to maxillæ in middle. A straight line on each side runs from articulation obliquely to base, dividing lip into three equal portions. *Maxillæ* wider in front, converging over lip. Outer angle pointed. Truncature short and scopula reaches proximally beyond it.

Serrula rather removed from margin, short, almost straight, not reaching outer angle. Upper surface of maxillæ yellow, lower brown with yellow end. *Sternum* convex, widely truncated in front to width of lip, slightly longer than wide in ratio 19:16. First coxæ separated by much more than width of lip, as much as third coxæ and but little less than second coxæ. Fourth coxæ contiguous. Sternum clothed with long black hair and is of the same color as lip and maxillæ. Legs almost of the same color, first and second pair slightly darker. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts well developed, grey. Claws slightly dissimilar, proclaw with a longer series of closer set teeth than retroclaw. *Legs* 1432.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.184	0.666	0.888	0.814	0.407	3.959
II	1.036	0.555	0.629	0.629	0.370	3.219
III	1.184	0.592	0.740	0.814	0.407	3.737
IV	1.073	0.518	0.777	0.962	0.444	3.774
Palp	0.555	0.296	0.148	0.555		1.554

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1 at end, elsewhere 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 0-1 bristle, ventral 2-2-2, first pair near base, elsewhere 0. Metatarsus—dorsal 0, prolateral 1 at end, retrolateral 0, ventral 2-2. *Second leg* same as first, except: Metatarsus—prolateral 1-1 retrolateral 0-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1 at end, retrolateral 1 at end. ventral 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 0-1 bristles, prolateral 1-1, retrolateral 1-1, ventral 1p-2. Metatarsus—dorsal 0, prolateral 1-2, retrolateral 1-2, ventral 2-2. *Fourth leg* same as third, except: Metatarsus—prolateral 1-1-2, retrolateral 1-1-2. *Palp* with 1 dorsal bristle on patella and several bristles on tibia and terminal joint. Legs clothed with black hair, with a few desultory white scales here and there. Distal end of femur and patella of palp thickly clothed with white scales on dorsal surface. Spinnerets yellow, cylindrical, equally long. Anterior pair slightly heavier. Colulus replaced by a small tuft of hair. Venter of abdomen dark brown with black hair. Sides with a narrow, longitudinal white stripe extending full length of abdomen. There are no scales on these stripes. Dorsum of abdomen with a white crescent across the entire front, covered with scales, and with a narrow white, procurved band

across abdomen. Rest dark, with long dark hair and a few bristles. Tibia of palp with a long, thin, obliquely curved apophysis. Femur of palp with a blunt protuberance in middle on inside.

c) Female from Cerro Flores. Total 4.8 mm. Carapace 2.009 mm. long, 1.435 mm. wide, 0.925 mm. high. Entire carapace very dark brown, shiny, with a few white scales on sides. Thoracic groove longitudinal. Its anterior end does not reach imaginary line tangent to posterior edge of PLE. Eyegroup 1.073 mm. long, thoracic region 1.184 mm. long. First row of eyes 1.369 mm. wide, second row 1.221 mm., third row 1.295 mm. PME nearer PLE than ALE in ratio 5:8. Ratio of eyes AME: ALE: PME: PLE = 11:7.5:1.75:6. Clypeus 1/4 of the diameter of AME, with a fringing row of fine, long bristles. Three bristles in a triangle on clypeus between AME and a pair of bristles on each side in front of ALE. *Chelicerœ* as in male and the tooth on retromargin quite sharp. *Lip* and sternum also as in male, but *maxillœ* with outer angle not produced and outer edge only slightly concave. *Legs* 4312.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	0.999	0.592	0.629	0.629	0.407	3.256
II	0.962	0.555	0.555	0.555	0.370	2.997
III	1.110	0.518	0.666	0.777	0.407	3.478
IV	1.221	0.518	0.777	0.888	0.444	3.848
Palp	0.481	0.296	0.259	0.481		1.517

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1 at end, elsewhere 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1-1 bristles, ventral 2-2-2, first pair near base, elsewhere 0. Metatarsus—ventral 2-2, elsewhere 0. *Second leg* same as first, except: Tibia—prolateral 1 at end, ventral 11-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 1 at end, retrolateral 0, ventral 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 1p-2. Metatarsus—dorsal 0, prolateral 1-2, retrolateral 1-2, ventral 2-2. *Fourth leg* same as third, except: Metatarsus—prolateral 1-1-2, retrolateral 1-1-2, ventral 1p-2. Epigynum shown in Figure 125. Abdomen above light grey-brown with a transverse, recurved, white band in front, reaching on sides to middle of abdomen; a white, slightly recurved transverse band in middle and 3-4 short, transverse, recurved, white bands behind. Venter with a dark field divided longitudinally into three bands

by two white bands, except near spinnerets where the three dark bands merge into one. Legs rather brilliantly yellow.

The generic position of *C. sulphurea* does not seem to be certain. The difficulty of placing this species properly in a genus is still increased by the differences in structure between the male and the female, as well as by the fact that in Simon's system closely allied species have been distributed among widely different genera. Is the shape of the tooth on the retromargin such an important character that similarities of other structures may be disregarded? And if so, are these similarities due to convergent development? These questions can be answered only after a thorough study of all characters and of the sequence of their appearance in the course of embryological and later development.

Corythalia panamana sp. nov. (Figures 129 and 130).

Male. Total with cheliceræ 7.68 mm. Carapace 3.744 mm. long, 2.784 mm. wide, 1.920 mm. high. Head flat. Thoracic groove longitudinal, its anterior end touching imaginary line tangent to posterior edge of PLE. First row of eyes 2.132 mm. wide, second row 2.050 mm., third row 2.132 mm. Eyegroup 1.804 mm. long, thoracic portion 2.460 mm. long. Ratio of eyes AME: ALE: PME: PLE = 17: 10: 3: 9. AME separated from each other by 2/17 of their diameter, from ALE by 3/17 of their diameter. PME nearer PLE than ALE in ratio 10.5: 13. PME separated from PLE by more than diameter of the latter. Clypeus 11/17 of diameter of AME. Viewed from in front, first row of eyes distinctly curved upward by upper margins. Edge of clypeus fringed with two rows of bristles. Three bristles in a triangle on clypeus in front of AME and a pair of bristles on each side in front of ALE. Each of the eyes of the first row surrounded by a circle of yellowish-grey scales. Head dark, covered with numerous black bristles and some white scales. White scales also scattered behind eyegroup and a broad band of white scales on each side reaching almost to margin. On the margin itself, however, the scales are dark. Rest of carapace with reddish-brown chitin clothed with black scales. *Chelicerœ* 1.110 mm. long, *i. e.* a fraction longer than face from upper edge of AME to edge of clypeus. Anteriorly cheliceræ are sparsely clothed with black, erect bristles and hair. Margins almost wanting. Promarginal end with a flap fringed with stiff, short hairs. Proximal to this flap an irregular

scopula. Retromargin with a single small tooth. *Lip, maxillæ* and *sternum* of the same type as in *C. spiralis*. Legs also of the same type, but fringe of black hair developed less on first leg and more on second and third. Trichobothria in two rows on all tibiæ, in 1 row on metatarsi and tarsi. Claws with a long series of teeth, those on the proclaw closer together than on the retroclaw. *Legs* 3412.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.214	1.295	1.332	1.221	0.666	6.728
II	2.214	1.258	1.369	1.184	0.666	6.691
III	2.542	1.295	1.591	1.887	0.814	8.129
IV	2.460	1.221	1.591	1.998	0.740	8.010
Palp	1.258	0.555	0.370	1.295		3.478

Legs clothed with black and white hair, with white scales scattered on all joints, but especially numerous on dorsal and

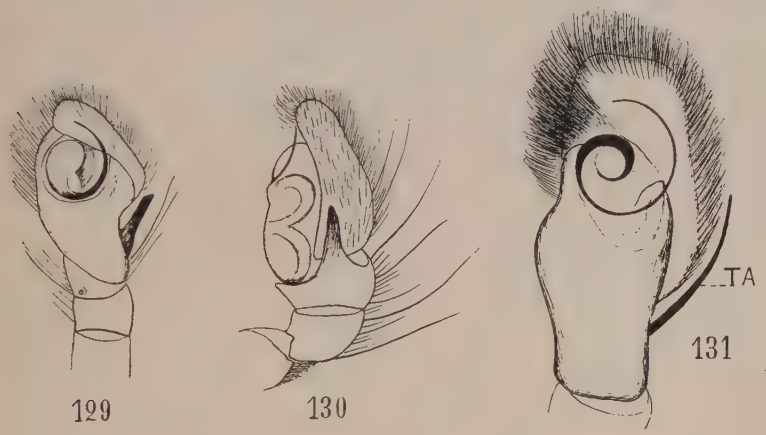


FIGURE 129, **Corythalia panamana** sp. nov., left palp from below. FIGURE 130, Same, left palp from outside. FIGURE 131, *Corythalia spiralis* (F. Cambridge), left palp from below.

lateral surfaces of femora. White scales also on dorsal surface of pedipalp, on distal end of femur and proximal end of patella.

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 2 at end, ventral 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1-1 bristles, prolateral 1-1, retrolateral 1-0, ventral 2-2-2, first pair near base. Metatarsus

—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2. *Second leg* same as first, except: Tibia—prolateral 1-1-1, ventral 1r-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 2 at end, ventral 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, the first a spine, the second a bristle, prolateral 1-1-1, retrolateral 1-1-1, ventral 1p-2. Metatarsus—dorsal 1-0, prolateral 1-2, retrolateral 1-2, ventral 1-1-2. *Fourth leg* same as third, except: Metatarsus—prolateral 1-1-2, retrolateral 1-1-2. *Abdomen* below clothed with short black hair and grey scales, above with very long black bristles, while the color and pattern are due to scales and hair. Anterior half of dorsum clothed with grey scales. Behind this area on each side a procurved band of black hair, then a procurved band of white scales, again a procurved band of black hair and close to the spinnerets once more white scales. Sides with a longitudinal band of white scales. Median spinnerets longest, posterior ones shortest, anterior pair heaviest. Colulus completely wanting. Legs dark brown, four posterior tarsi yellow. Sternum, lip, maxillæ, coxæ and trochanters yellow. Tibial apophysis of palp much shorter and stouter than in *C. spiralis.* Cymbium large and bulky. Embolus completing one turn of a spiral. Habitat: Remedios and Panama City.

Corythalia spiralis (F. Cambridge), (Figure 131).

Male. Total with cheliceræ 8.90 mm. Carapace 4.224 mm. long, 3.072 mm. wide, 1.824 mm. high. Head slightly convex. Thoracic groove longitudinal, scarcely visible; its anterior end does not reach the imaginary line tangent to posterior edge of PLE. First row of eyes 2.275 mm. wide, second row 2.275 mm., third row 2.275 mm. Eyegroup 1.837 mm. long, thoracic portion 2.800 mm. long, *i. e.* is much longer than the eyegroup. PME nearer PLE than ALE in ratio 9:13. Ratio of eyes AME: ALE: PME: PLE = 19:11:3.75:10.5. AME separated from each other by 2/19 of their diameter, from ALE by 3/19 of their diameter. Clypeus 13/19 of the diameter of AME, fringed with bristles. Three bristles in a triangle on clypeus between AME and a pair of bristles on each side in front of ALE. Head with numerous black bristles and white scales. An angular, narrow band of white bristles extends on each side from between the PLE to the place where the posterior declivity begins. Elsewhere cara-

pace clothed with recumbent, black hair. Sides with a wide band of white scales. *Chelicerœ* stout and short and so directed that the fangs form a straight line transverse to the plane of symmetry. Margins almost wanting. Promargin with a flap fringed with stiff, short, black bristles which form a regular comb. Proximal to the flap an irregular scopula. Retromargin with a single, small tooth. Anteriorly chelicerœ are sparsely clothed with white hair. The chitin here is very dark brown; it is lighter below and almost yellow on inside. *Lip* is almost triangular, very narrow in front, longer than wide at base in ratio 20:17, articulated to maxillæ in middle and reaching beyond middle of maxillæ. The lip is light brown with yellow tip. *Maxillœ* distinctly converging, rounded at end to such an extent that no real truncature is distinct, but the thick scopula extends from the middle of the distal end along the inner edge as far down as the lip. Outer angle strongly produced and outer margin concave. Serrula almost, though not quite marginal, short, not reaching outer angle. Maxillæ of the same color as lip, with yellow inner edge and tip. *Sternum* quite flat, longer than wide in ratio 45:32, narrowed anteriorly to width of lip. First coxæ separated by more than width of lip, but less than third coxæ. Fourth coxæ contiguous. Sternum, coxæ and trochanters clothed with long, erect, black hair and of the same color as the lip and the maxillæ. Legs dark brown to black, only fourth pair and third tarsi much lighter. Legs clothed with black hair and with white scales scattered over all joints. First, second and third tibiæ with purple iridescence. A long dorsal and ventral brush of black hair on first, second and especially on third patella, tibia and metatarsus. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts very thick. Claws with a long series of teeth, similar on all tarsi. *Legs* 3412.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.460	1.599	1.558	1.353	0.738	7.708
II	2.460	1.558	1.476	1.394	0.738	7.626
III	2.952	1.558	1.886	1.968	0.943	9.307
IV	2.788	1.353	1.845	2.214	0.943	9.143
Palp	1.148	0.492	0.287	1.148		3.075

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1-1, ventral 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1 bristles, prolateral 1-2, retrolateral 1-1, ventral 2-11-2, first pair near base.

Metatarsus—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2. *Second leg* same as first, except: Tibia—prolateral 1-1-1, ventral 2-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-2, retrolateral 1-2, ventral 0. Patella—dorsal 1-1, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1 bristles, prolateral 1-1-1, retrolateral 1-1-1, ventral 2-2. Metatarsus—dorsal 0, prolateral 1-2, retrolateral 1-2, ventral 2-2. *Fourth leg* same as third, except: Femur—retrolateral 0-2. Tibia—dorsal 1-1, the first a spine, the second a bristle. Metatarsus—prolateral 1-1-2, retrolateral 1-1-2. *Palp* clothed with white hair and white scales on femur and patella. There are also four black bristles on the patella. Tibia and terminal joint only with black hair and black bristles. Bulb yellow below. Tibial apophysis exceedingly long and fine. Embolus spiral, completing more than one and one half turns. Abdomen yellow with grey mottling below, the color due entirely to the chitin, clothed with black hair of varying length. On the back the color of the abdomen is due to hair and scales and is very striking. Long, erect, black bristles are scattered all over the back between the hair and the scales. A large, fiery red spot in middle, followed by a transverse bar of white scales, which is interrupted in the middle. A pair of oblique, narrow, white bands in the posterior quarter and two pairs on the sides in front of the red spot. The white in all cases is due to scales, the red to hair. An area of slate grey scales in the middle in front and behind the red spot. The rest of the back and the sides are clothed with black hair. Spinnerets brown, about equal in length, cylindrical, anterior pair heaviest. Colulus replaced by a few hairs. Habitat: Panama, Costa Rica, Guatemala and Mexico.

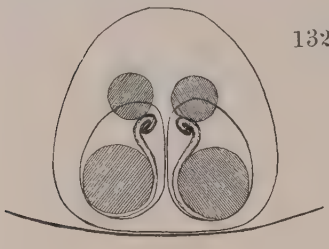
Corythalia bicincta sp. nov. (Figures 132 and 133).

a) Male. Total 4.608 mm. Carapace 2.296 mm. long, 1.681 mm. wide, 1.230 mm. high. Thoracic groove longitudinal, its anterior end slightly in advance of imaginary line tangent to posterior edges of PLE. Eyegroup 1.147 mm. long, thoracic portion 1.295 mm. long. First row of eyes 1.517 mm. wide, second row 1.332 mm., third row 1.406. PME nearer to PLE than to ALE in ratio 6:8, separated from PLE by less than diameter of the latter. Ratio of eyes AME:ALE:PME:PLE = 12:8:2:7. AME distinctly separated from each other, though very close together, and slightly more separated from ALE. Clypeus 7/12

of the diameter of AME. Viewed from in front, first row of eyes slightly curved upward by upper margins. AME in a circle of scales which are white in the lateral quadrants of the circle, but dark in the upper and lower quadrant. A semicircle of white scales below ALE. Clypeus with two transverse bands of white scales, first band forming the fringe on the edge of the clypeus, second band above it, just below the bristles. There is also a transverse band of white scales on the anterior surface of the cheliceræ near base. On viewing the spider from in front, the first impression is therefore that the face has three transverse white bands. The cheliceral scales seem to be easily rubbed off in life, in which case only the two clypeal white bands remain. Three bristles in a triangle on clypeus between AME and a pair of bristles on each side in front of ALE. Bristles on head numerous, of variable size. A semicircle of white scales at base of each PLE to the inside of it, the right and left one in contact with each other in front of thoracic groove. Posterior declivity very steep, but begins considerably behind thoracic groove. Sides of carapace opposite second and third leg with a wide band of white scales, reaching almost to margin. *Cheliceræ* rather weak, with a very small, stout fang. Promargin with a flap fringed with 6 short, stiff bristles, followed proximally by some chitinous granules, but real teeth wanting. Retromargin with a very small, sharp tooth. There is some hair along each margin, that on the retromargin forming a single row. *Lip* triangular, as long as wide, reaching considerably beyond middle of maxillæ, truncated at end, articulated to maxillæ 1/3 from base. *Maxillæ* inclined over lip, truncatures very strongly divergent and in almost even continuation with anterior margin. Outer angle barely indicated and outer edge short and straight. Scopula reaching level of lip. Serrula not marginal and does not reach outer angle. *Sternum* fairly flat, oval, broadly truncated in front to a little more than width of lip, longer than wide in ratio 26:18. First coxæ separated by 1.5 the width of the lip. Legs hairy, the hair black. A long black fringe of hair on the dorsal and ventral surface of patella, tibia and metatarsus of first, second and especially of third leg. White scales on almost all joints, but not numerous. Trichobothria in two rows of two each on all tibiæ, in one row of 5 or 6 on metatarsi increasing in length distally, and in one row of 4 or 5 on tarsi, also increasing in length distally. Ungual tufts thick.

Claws slightly dissimilar. Proclaw with a long row of closely set teeth, retroclaw with fewer teeth set farther apart, the proximal ones quite small, almost in the shape of granules. *Legs* 4321.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.258	0.666	0.851	0.777	0.518	4.070
II	1.295	0.629	0.851	0.814	0.518	4.107
III	1.554	0.777	1.110	1.184	0.555	5.180
IV	1.591	0.666	1.110	1.332	0.555	5.254
Palp	0.629	0.370	0.200		0.666	1.865



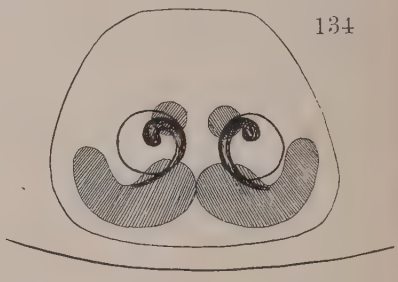


FIGURE 132, **Corythalia bicincta** sp. nov., epigynum. FIGURE 133, Same, left palp from below. FIGURE 134, **Corythalia pulchra** sp. nov., epigynum. FIGURE 135, Same, dorsal view of abdomen.

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1 at end, retrolateral 1 at end, ventral 0. Patella 1-1-1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1-1-1 bristles, prolateral 1-1, retrolateral 0, ventral 1r-2-2. Metatarsus—dorsal 0, prolateral 1-1, retrolateral 0-1, ventral 2-2. *Second leg* same as first, except:

Femur prolateral 2 at end, retrolateral 2 at end. Patella—retrolateral 1. Tibia—prolateral 1-1-1, retrolateral 0-1. Metatarsus—retrolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1-1, first one a spine, second and third bristles, prolateral 1-1-1, retrolateral 1-1-1, ventral 1-2. Metatarsus—dorsal 1r near base, prolateral 1-2, retrolateral 1-2, ventral 2-2. *Fourth leg* same as third, except: Metatarsus—dorsal 1r-1r, prolateral 1-1-2, retrolateral 1-2. *Palp.* Two dorsal bristles on patella. End of femur and patella thickly clothed with white scales on dorsal surface. Tibial apophysis long, straight. Embolus completing exactly one turn of the spiral. *Abdomen* sparsely clothed with short black hair below, while above it is clothed with hair and scales as follows: A wide band of yellow scales extends across the entire front, continues on sides diverging as far as middle where a procurved arc across the middle is formed. The area inside this band is clothed with long black hair, posteriorly to the yellow band three transverse yellow bands are found on the back of the abdomen. The first of these is quite short, the second still shorter and connected with the third which is wide enough to reach almost to the sides of the abdomen. The area between these bands and laterally to them is clothed with long, black hair. Black, long bristles are numerous on the abdomen and equally distributed over the yellow bands and the dark areas. Spinnerets cylindrical, long and slender, all of about the same length, but anterior pair stouter. The median pair is situated between the spinnerets of the posterior pair. Anterior spinnerets are brown, the others light yellow. Colulus wanting. Legs dark, especially the first pair. Third tarsi yellow. Size, coloration, structure of palp and the white fringes on clypeus and cheliceræ make the recognition of this species simple.

b) Female. Total 5.56 mm. Carapace 2.132 mm. long, 1.640 mm. wide, 1.189 mm. high. Thoracic groove longitudinal. Shape of carapace and distribution of scales and bristles about the same as in male. First row of eyes 1.554 mm. wide, second row 1.369 mm., third row 1.406 mm. PME much nearer PLE than ALE in ratio 5.5:9. PME separated from PLE by less than the diameter of the latter. Ratio of eyes AME: ALE: PME: PLE = 13:8:2.5:7. Clypeus 6/13 of the diameter of AME with the

characteristic two rows of white scales. Cheliceræ, lip, maxillæ and sternum as in male. *Legs* 4321.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.258	0.703	0.703	0.703	0.444	3.811
II	1.258	0.703	0.703	0.703	0.444	3.811
III	1.480	0.740	0.888	1.036	0.518	4.662
IV	1.480	0.703	1.036	1.184	0.518	4.921
Palp	0.629	0.296	0.275	0.592		1.792

Fringe of long hair, such as in male, is wanting on all legs.

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, elsewhere 0. Patella—dorsal 1-1-1-1 bristles, the distal one alone erect and long; prolateral 1, elsewhere 0. Tibia—dorsal 1-1-1 bristles, the distal one alone erect and long; prolateral 1-1, retrolateral 0, ventral 0-2. Metatarsus—dorsal 0, prolateral 1-1, retrolateral 0, ventral 2-2. *Second leg* same as first, except: Tibia —prolateral 1-1-1, ventral 1r-2. Metatarsus—retrolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1-1-1 bristles, first and fourth erect and long; prolateral 1, retrolateral 1, ventral 0. Tibia —dorsal 1-1-1, the first a short spine, the second and third bristles; prolateral 1-1-1, retrolateral 1-1-1, ventral 1-2. Metatarsus— dorsal 1 near base, prolateral 1-2, retrolateral 1-2, ventral 2-2. *Fourth leg* same as third, except: Metatarsus—prolateral 1-1-2, retrolateral 1-1-2. Lip, maxillæ, sternum, coxæ, trochanters and base of femora yellow. Rest of legs brown. Distal end of femur and entire patella of palp yellow. Coloration of abdomen much as in male, but the central yellow transverse band straight and wider in middle where it forms a "diamond" more or less confluent with a yellow spot in front and the short transverse yellow band behind. Habitat: Wilcox camp on San Lorenzo River.

Corythalia pulchra sp. nov. (Figures 134 and 135).

Female. Total with spinnerets 6.432 mm. Carapace 2.706 mm. long, 1.927 mm. wide, 1.394 mm. high. Abdomen without spinnerets 2.870 mm. long, 2.255 mm. wide in region of transverse band. Posterior declivity of carapace forms with the longitudinal axis an angle of 60°. Thoracic groove longitudinal, its anterior end touching the imaginary transverse line tangent to posterior edges of PLE. Eyegroup 1,184 mm. long, thoracic portion

1.850 mm. long. First row of eyes 1.628 mm. wide, second row 1.554 mm., third row 1.628 mm. PME nearer PLE than ALE in ratio 7 : 10. PME separated from PLE by the diameter of the latter. Ratio of eyes AME : ALE : PME : PLE = 13 : 8 : 2.25 : 7. Clypeus 7.5/13 of the diameter of the AME. AME separated from each other by 1.5/13 of their diameter, from ALE by 2/13 of their diameter. Edge of clypeus fringed with a row of white scales. Above this row a row of bristles among which two pairs are especially large. Three bristles in a triangle on clypeus between AME and a pair of bristles on each side in front of ALE. First row of eyes curved upward by upper margins, when viewed from in front, considerably recurved when viewed from above. Line tangent to anterior margins of ALE passes behind base of AME. Head flat. Bristles on head numerous, of varying size. A patch of white scales between ALE occupies almost the entire width of the head. A small patch of white scales on each side of head between PME and PLE, forming a procurved semicircle behind the former. A patch of white scales on each side on the thoracic portion a little behind PLE. A wide band of white scales on each side of carapace extending almost to margin. Main portion of head clothed with greyish-yellow scales. Thoracic declivity clothed with black hair. *Cheliceræ* stout and short, parallel. Their base clothed with white scales and hair. Promargin with a flap fringed with 6 short, stiff hairs, followed proximally by a small tooth. Retromargin with a very small, sharp tooth. Some hair along promargin, and some on retromargin where it forms one row. *Lip* neatly triangular with slightly curved sides, a little longer than wide in ratio 12 : 11. It reaches considerably beyond middle of maxillæ and is articulated to them one-third from base. *Maxillæ* distinctly inclined over lip. Truncature and upper margin form a single curve. Outer angle rounded and outer edge straight. Scopula dense and of short hair, reaches the lip. Serrula short and almost marginal. *Sternum* longer than wide in ratio 27 : 20, flat, truncated anteriorly to the width of the lip, with three distinct angles on each side opposite intercoxal spaces, and a pair of angles at posterior end. First coxæ separated by much more than the width of the lip and as much as third coxæ. Fourth coxæ contiguous. First and second pair of legs distinctly heavier than third and fourth. All joints clothed with long black hair

and with occasional white scales which are especially apparent on the dorsal surface of the femora and are completely wanting on the tarsi. Ungual tufts well developed, grey. Claws somewhat dissimilar. Proclaw with a long series of closely set teeth, retroclaw with not as many teeth. *Legs* 4321.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.295	0.814	0.740	0.740	0.444	4.033
II	1.369	0.814	0.740	0.740	0.481	4.144
III	1.554	0.740	0.925	1.073	0.518	4.810
IV	1.628	0.777	1.036	1.258	0.555	5.254
Palp	0.703	0.370	0.333	0.629		2.035

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1-1-1 bristles, first and fourth erect and long; prolateral 1, elsewhere 0. Tibia—dorsal 1 bristle at end, prolateral 1-1, retrolateral 0, ventral 1r-1r-2. Metatarsus—dorsal 0, prolateral 1-1, retrolateral 1-1, ventral 2-2. *Second leg* same as first, except: Femur—retrolateral 2 at end. Tibia—prolateral 1-1-1, retrolateral 1-0. *Third leg.* Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 2 at end, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, first a spine, second a bristle; prolateral 1-1-1, retrolateral 1-1-1, ventral 1p-2. Metatarsus—dorsal 1r near base, prolateral 1-1-1-1, retrolateral 1-1-2, ventral 1p-2-2. *Fourth leg* same as third, except: Metatarsus—prolateral 1-1-2, retrolateral 1-1-2, ventral 0-2-2. *Palp* has no spines, but a long middorsal apical bristle on patella and numerous bristles on tibia and on terminal joint. Palpal claw wanting. *Abdomen* thickly clothed with hair and scales and with numerous long, black bristles. The dark portions shown in Figure 135 are clothed entirely with recumbent, black hair directed backward and inward. The middorsal longitudinal band with yellow scales which are found also on the sides of the abdomen. The transverse bands and other light spots are clothed with white scales. Venter sparsely clothed with the usual short black hair and long, erect, black hair. Spinnerets of equal length, but anterior pair the heaviest. Median spinnerets situated on a common elevation, between the posterior pair. Anterior spinnerets brown, median

pair very light yellow, posterior pair yellow mottled with brown. Colulus wanting. Epigynum with small, circular openings. Posterior receptacles bean-shaped. The ducts are curved and divergent. The species is in many respects similar to *C. bicincta*, but distinct from it. Habitat: Panama City, Fort Sherman, C. Z.

Beata magna Peckham (Figure 136).

Female. Total 6.75 mm. Carapace 2.500 mm. long, 2.255 mm. wide, 1.066 mm. high, sloping gently forward, with head quite flat. Posterior declivity steep. Thoracic groove longitudinal, somewhat concealed by scales. Between carapace and coxæ a chitinized

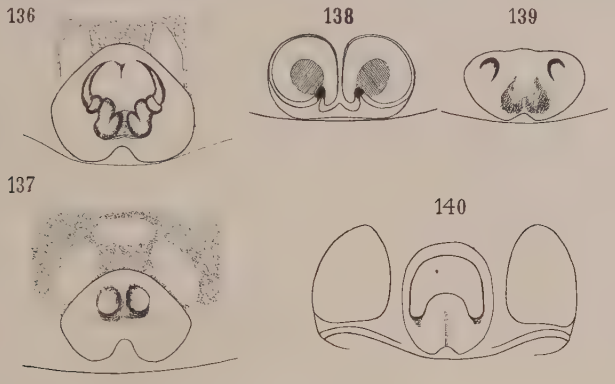


FIGURE 136, *Beata magna* Peckham, epigynum. FIGURE 137, **Beata striata** sp. nov., epigynum. FIGURE 138, *Chapoda inermis* (F. Cambridge) epigynum. FIGURE 139, **Zygoballus maculatipes** sp. nov., epigynum. FIGURE 140, *Breda milvina* (C. Koch), epigynum.

epimerite extending uninterruptedly from end to end and clothed with white scales. First row of eyes 1.804 mm. wide, second row 1.681 mm., third row 1.886 mm. Eyegroup 1.394 mm. long. Ratio of eyes AME:ALE:PME:PLE = 15:8:2:7.5. AME sub-contiguous at base and sub-contiguous with ALE. PME nearer ALE than PLE in ratio 8:12. PME separated from ALE by the diameter of the latter. When viewed from in front first row of eyes curved upward by upper margins, and still more so by centers. Clypeus 2/15 of the diameter of AME. Except for the median area on the posterior declivity, carapace clothed with

white scales and black hair. Two bristles, one behind the other, on clypeus in median line; several bristles behind AME and ALE. A pair of bristles to the outside of PLE on each side of the head and two bristles, one behind the other, behind each PLE. Clypeus fringed with long, white hair. White scales abundant on cheliceræ, palpi and legs, on dorsal surface of abdomen and even on sternum. *Cheliceræ* strongly geniculate at base, flat in front, rather powerful, with gently oblique margins. Promargin with two small teeth and a black scopula. Retromargin with a strong, typically bicuspid tooth. Fang short and strong. *Lip* longer than wide in ratio 14:13, considerably narrowed toward end, articulated to maxillæ a little before middle. Lip reaches beyond middle of maxillæ. *Maxillæ* with short truncature bearing a thick scopula. Outer angle rounded. Serrula long, reaching over outer angle. *Sternum* longer than wide in ratio 29:21, oval, truncated in front, rounded behind, convex. First coxæ separated by width of lip, second coxæ widest apart, fourth coxæ contiguous. First leg considerably heavier, second somewhat heavier than the third and fourth. First femur strongly convex dorsally, flattened laterally and ventrally. Second femur of the same type though less convex above or flattened below and on sides. First and second tibia cylindrical and much heavier than metatarsus. All tarsi much heavier at end than at base. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts very thick. Claws strongly curved, with numerous teeth increasing in length distally. Palpal claw wanting. *Legs* 1423.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.554	1.073	1.036	0.925	0.555	5.143
II	1.406	0.814	0.777	0.814	0.481	4.292
III	1.480	0.777	0.740	0.777	0.481	4.155
IV	1.729	0.777	0.962	1.036	0.518	5.022
Palp	0.925	0.370	0.407	0.999		2.701

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, elsewhere 0. Patella—dorsal 1-1 bristles, elsewhere 0. Tibia—dorsal 0-1 bristle, ventral 2-2-2, first pair more than 1/3 from base, elsewhere 0. Metatarsus—ventral 2-2, first pair beyond middle, elsewhere 0. *Second leg* same as first, except: Tibia—ventral 1r-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 1 at end, elsewhere 0. Patella—dorsal 1-1 bristles, elsewhere 0. Tibia—

dorsal 0-1, prolateral 1, retrolateral 0, ventral 0-2. Metatarsus—dorsal 0, prolateral 0-2, retrolateral 0-2, ventral 0-2. *Fourth leg* same as third, except: Femur—prolateral 0, retrolateral 0-1. Tibia—prolateral 0, retrolateral 1. Metatarsus—prolateral 0-1. *Palp.* Femur—dorsal 0-1, elsewhere 0. On other joints only bristles, as follows: Patella—dorsal 1-1. Tibia—dorsal 1, prolateral 1-1, retrolateral 1. Terminal joint—dorsal 1. Habitat: Panama, Guatemala, Colombia.

Beata striata sp. nov. (Figure 137).

Female. Total 4.715 mm. Carapace 2.035 mm. long, 1.776 mm. wide, 0.999 mm. high. Thoracic groove small, longitudinal. Head flat, granular, shiny, with green iridescence. Posterior declivity begins as far behind third row of eyes as this row is from first row. First row of eyes 1.406 mm. wide, second row 1.369 mm., third row 1.591 mm. Eyegroup 1.147 mm. long. Ratio of eyes AME:ALE:PME:PLE = 12:6.5:1.5:6. PME farther from PLE than from ALE in ratio 9:8. Viewed from in front, first row of eyes is straight by upper margins, curved upward by centers. AME sub-contiguous, separated from ALE by 2/12 of their diameter. ALE and PLE in a black field deeply constricted behind PME. Clypeus equal to the radius of AME, thickly clothed with white lanceolate hair. Carapace clothed with white scales. 1-1 median bristles on clypeus; a semicircle of bristles behind each AME; 2 bristles to inside of each AME; 1 bristle behind each PME, 2 to outside of each PLE and 1-1 bristles behind each PLE. *Cheliceræ* geniculated at base, stout and rather short, clothed with scales in front. Margins moderately oblique. Promargin with two teeth and a few hairs in place of a scopula. Retromargin with a strong, typically bicuspid tooth. Fang short, curved, normal. *Lip* longer than wide in ratio 11:10, gradually narrowed toward end where it is truncated; articulated to maxillæ in middle; reaching slightly beyond middle of maxillæ. *Maxillæ* evenly rounded at outer angle. Truncature short, with brown scopula. Serrula moderately long, reaching middle of outer angle. *Sternum* longer than wide in ratio 22:15, narrowed in front to width of lip and truncated. First pair of coxæ separated by the width of the lip, fourth pair contiguous. First pair of legs considerably heavier, second pair slightly heavier than third and fourth. First femur convex dorsally, moderately convex ventrally,

somewhat flattened on sides. Tibia cylindrical. Metatarsus much more slender than tibia. All tarsi wider at end than at base. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Claws strongly curved, with a long row of teeth. Palpal claw wanting. *Legs* 1423.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.406	0.962	1.036	0.740	0.555	4.699
II	1.110	0.740	0.703	0.666	0.481	3.700
III	1.110	0.592	0.666	0.629	0.481	3.478
IV	1.406	0.740	0.851	0.814	0.518	4.329
Palp	0.740	0.296	0.370	0.444		1.850

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, elsewhere 0. Patella—dorsal 1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1-1 bristles, ventral 3-3, first proventral spine almost in middle, first retroventral spine about one-quarter from base, elsewhere 0. Metatarsus—ventral 2-2, elsewhere 0. *Second leg* same as first, except: Femur—retrolateral 1 at end. Tibia—ventral 1r-1r-2, prolateral 1. Metatarsus—prolateral 1-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1 bristles, retrolateral 1, elsewhere 0. Tibia—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0-2. Metatarsus—dorsal 0, prolateral 1-2, retrolateral 1-2, ventral 0-2. *Fourth leg* same as third, except: Tibia—prolateral 0, ventral 1p-2. *Palp.* Femur—dorsal 1-1, elsewhere 0. Patella—dorsal 1-1 bristles, elsewhere 0. Tibia—dorsal 1-1 bristles, elsewhere 0. Terminal joint—dorsal 1 bristle. Femur of palp considerably curved both downward and inward. *Abdomen* 2.46 mm. long, 1.599 mm. wide. Spinnerets about equal in length. Median and posterior pair cylindrical, anterior pair distinctly cone-shaped. Colulus wanting. Epigynum flat, with two oval foveæ. Color in alcohol: Head reddish with green iridescence, rest of carapace of the same color, but obscured by white scales. A pair of longitudinal, parallel, black bands formed by the confluent black areas of the eyes. Cylindrical base of AME brown. Abdomen yellow above with four pairs of brown spots and smaller, irregular brown spots and stripes. Abdomen sparsely clothed with simple hair. Cheliceræ, maxillæ and sternum red-brown, lip darker. Palpi yellow. White scales on all femora. First leg dark red-brown with lighter spots on femur, patella and tibia; metatarsus and

tarsus lighter. The other legs yellow with darker spots on femur, patella and tibia. Sides of abdomen striped obliquely with brown. Abdomen below yellow with light brown field in posterior half. Spinnerets brown. Habitat: Cerro Iglesia.

Chapoda inermis (F. Cambridge) (Figure 138).

Female. Total 5.952 mm. Carapace 2.706 mm. long, 1.968 mm. wide, 1.435 mm. high. Thoracic groove longitudinal, in a slight depression. First row of eyes 1.924 mm. wide, second row 1.702 mm., third row 1.776 mm. Eyegroup 1.554 mm. long, *i. e.* much wider than long in ratio 52:42. Ratio of eyes AME:ALE: PME:PLE = 16:9:1.75:8. AME contiguous, and sub-contiguous with ALE. PME before middle, nearer PLE than ALE in ratio 8:10, if the distance between PME and ALE is measured not to the base of the cylinder of the latter, but to the eye itself. Clypeus equal to one half of the radius of AME, fringed with white scales. Head flat. Three bristles in a triangle on clypeus with apex directed posteriorly, and 1 bristle on each side in front of AME. Several bristles behind each anterior eye and some bristles between and behind eyes of second and third row. All eyes surrounded by circles of white and yellow scales. A group of white scales between ALE and AME, and between AME. *Cheliceræ* strong, but not long, with moderately oblique margins. Promargin with 2 teeth and a thin scopula. Retromargin with a single, typically bicuspid tooth, and a few hairs on retrosurface. Fang short, normal, serrated on ventral retroedge in distal two-thirds. *Lip* longer than wide in ratio 12:10, articulated to maxillæ in middle, rounded anteriorly, reaching but little beyond middle of maxillæ. *Maxillæ* slightly converging; wider anteriorly; with short, strongly diverging truncature and evenly rounded outer angle. Scopula thick, dark, occupying truncature and continuing downward along inner edge where it is of light color. Serrula reaching beyond middle of outer angle. *Sternum* longer than wide in ratio 29:21, narrowed anteriorly to width of lip and truncated. Clothed with fine, long, erect hair. A stiff, long bristle opposite each coxa including palp, but excepting fourth coxæ. First pair of coxæ wide apart, fourth contiguous. Legs sparsely clothed with dark simple hair. Trichobothria in two rows on all tibiæ, in one row on all metatarsi and tarsi. Claws dissimilar.

Proclaw with numerous fine teeth, retroclaw with only 4 or 5 teeth. Palpal claw wanting. *Legs* 4312.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.369	0.925	0.999	0.851	0.518	4.662
II	1.406	0.814	0.851	0.777	0.481	4.329
III	1.776	0.851	0.999	1.110	0.592	5.328
IV	1.702	0.777	1.110	1.369	0.555	5.513
Palp	0.740	0.370	0.370	0.592		2.072

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, elsewhere 0. Patella—dorsal 1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1-1-1 bristles, prolateral 1-1-1, retrolateral 0-1, ventral 2-2-2, first pair near base. Metatarsus—dorsal 1, prolateral 1 at end, retrolateral 1 at end, ventral 2-2-2, second pair shortest, first pair reaching beyond base of third. *Second leg* same as first, except: Femur—retrolateral 1 at end. Patella—retrolateral 1. Tibia—ventral 1r-2-2, second pair small and easily mistaken for laterals. *Third leg.* Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1 at end, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1-1, the first a short spine, the second and third bristles, prolateral 1-1-1, retrolateral 1-1-1, ventral 1p-0-2. Metatarsus—dorsal 1r-0, prolateral 1-2, retrolateral 1-2, ventral 2-2. *Fourth leg* same as third, except: Metatarsus—ventral 1p-1. *Palp.* Femur—dorsal 1-1. Patella—dorsal 1-1 bristles. Tibia—dorsal 1-1-1 bristles. Terminal joint with several irregularly arranged bristles. No bristles or spines elsewhere on palp. *Abdomen* clothed with black hair and iridescent orange scales. Spinnerets equal in length, but anterior pair heaviest and distinctly cone-shaped. Colulus replaced by a tuft of hair. Anal tubercle large. Epigynum very flat and transparent, with receptacles visible through the integuments. Color in alcohol: Carapace dark yellow with black around posterior eyes. Chelicerae dark yellow. Lip and maxillae paler. Rest of spider light yellow. Back of abdomen with a pair of longitudinal dark bands and a few transverse dark lines behind. Habitat: Costa Rica, Mexico and Panama.

Zygoballus maculatipes sp. nov. (Figure 139).

Female. Total 3.485 mm. Carapace 1.628 mm. long, 1.332 mm. wide, 0.851 mm. high. First row of eyes 1.147 mm. wide, second row 0.999 mm., third row 1.332 mm., giving a ratio for I : II : III

= 31:27:36. Eyegroup 0.999 mm. long, longer than posterior declivity. Carapace highest in region of PLE, head flat. Posterior declivity forms with the head an angle of 65°, with the base of the carapace an angle of 45°. Thoracic groove entirely concealed by scales. Viewed from in front, first row of eyes straight by upper margins. PME situated nearer ALE than PLE in ratio 7:9. Ratio of eyes AME:ALE:PME:PLE = 10:5.5:1.25:5. Thoracic portion of carapace 0.814 mm. long. Posterior edge strongly emarginate. Except for a triangle on posterior declivity, carapace clothed with white scales and fine bristles. Anterior four eyes contiguous. Clypeus practically wanting. The edge of the carapace fringed with white scales which are also present on cheliceræ and femora. *Cheliceræ* stout, with oblique margins. Promargin with 2 teeth and a scopula composed of a single row of hairs. Retromargin with a single, typically bicuspid tooth and a few hairs. Fang normal, with a distinctly serrated retroedge. *Lip* as long as wide, gradually narrowed toward end where it is truncated. *Maxillæ* inclined over lip, outer angle rounded, short truncature with thick scopula. Serrula reaching middle of outer angle. *Sternum* longer than wide in ratio 18:13, narrowed in front to width of lip, pointed behind, oval. First coxæ separated by width of lip, fourth coxæ contiguous. First pair of legs heaviest. Femur convex dorsally, flattened on sides and ventrally. Tibia cylindrical. Metatarsus much more slender than tibia. Other legs normal, but third and fourth trochanters notched. *Legs* 4123.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	0.925	0.555	0.740	0.481	0.370	3.071
II	0.740	0.407	0.481	0.412	0.333	2.373
III	0.666	0.370	0.444	0.444	0.370	2.294
IV	1.073	0.518	0.740	0.666	0.407	3.404
Palp	0.481	0.222	0.222		0.333	1.258

Spines. First leg. Femur—dorsal 1-1-1, prolateral 1, elsewhere 0. Patella—dorsal 0-1 bristle, elsewhere 0. Tibia—dorsal 1-1 bristles, ventral 2-2-2, first pair near base, elsewhere 0. Metatarsus—ventral 2-2, elsewhere 0. *Second leg* same as first, except: Femur—dorsal 0-1-1, prolateral 0. Tibia—ventral 1-1. *Third leg.* Femur—dorsal 1-1, elsewhere 0. Patella—dorsal 0-1 bristle, elsewhere 0. Tibia—dorsal 1-1 bristles, prolateral 0-1, else-

where 0. Metatarsus—dorsal 0, prolateral 0-1, retrolateral 0-2, ventral 0-1. *Fourth leg* same as third, except: Femur—dorsal 1-1-1. Tibia—ventral 1p-1p-2. Metatarsus—ventral 1-1. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Claws dissimilar. Proclaw with a long row of teeth, retroclaw with but 3 strong teeth. Ungual tufts comparatively weak. Abdomen clothed with simple and with lanceolate hair. Spinnerets of equal length, but anterior pair heavier. Colulus replaced by some hair. Epigynum with a pair of openings. Color in alcohol: Carapace reddish-brown with green iridescence and with white lanceolate hair. Palpi light yellow. Cheliceræ reddish-brown, lighter than carapace. Lip, maxillæ and sternum of the same color. First trochanters and femora somewhat darker than the others. First, second and third coxæ light yellow with a small black spot; fourth coxæ dark brown with a light yellow spot. Second, third and fourth trochanters yellow. First patella and tibia yellow, with a proventral dark band, first metatarsus light yellow, first tarsus very dark brown. Second leg yellow with a black band on prolateral surface of femur, patella and tibia, and three spots on retrolateral surface of femur. Third leg much as second, but the dark more extensive, so that the tibia is entirely black except on ventral surface. Fourth leg with dark brown femur. This color is replaced by yellow only near base, above. Patella yellow with brown ends, tibia brown except on dorsal surface where there is some yellow, metatarsus yellow with brown end, tarsus brown. Abdomen reddish-brown above with three pairs of yellow spots. An irregular yellow longitudinal band on each side. Venter brown stippled with black. Habitat: Wilcox camp on San Lorenzo River.

Breda milvina (C. Koch) (Figure 140).

Female. Total 15.0 mm. Carapace 4.800 mm. long, 3.648 mm. wide, evenly oval, widest in middle, much wider than eyegroup, flat, slightly depressed behind PLE. Thoracic groove longitudinal, small, well-visible, although covered with hair. Eyegroup shorter than rest of carapace in ratio 24:26. PLE far removed from edge. Width of eyerows in ratio I:II:III = 65:58:63. PME situated in middle between ALE and PLE. Ratio of eyes AME:ALE:PME:PLE = 22:11:2:11. AME sep-

arated from each other by 3.5/22 of their diameter, from ALE by 5/22. Viewed from in front, first row of eyes is straight by upper margins, curved upward by centers. Clypeus equal to 5/22 of the diameter of AME. Arrangement of bristles on carapace irregular. Head black with purplish-green iridescence. A median band of recumbent, white hair extends from eyegroup to posterior edge of carapace. Margins with a narrow band of white hair. Surface of thoracic portion between median and lateral bands dark red-brown with a few erect, black hairs. A pair of black bristles between AME in line with the lower edge of the eyes. Behind this pair a median black bristle. Edge of clypeus with a row of long, white hairs concealing base of cheliceræ. *Cheliceræ* short, almost black with corrugated anterior surface. Boss wanting. Margins moderately oblique. Promargin with 4 teeth. Retromargin with a single small tooth opposite the fourth promarginal tooth. Scopula on promargin only. Fang small, stout, evenly curved. *Lip* dark brown, longer than wide in ratio 25 : 19, gradually narrowed toward end where it is truncated and bears long, parallel, black hair. *Maxillæ* dark brown, wider anteriorly, with outer angle rounded. Truncature short, yellow. The two truncatures form between them an angle of 70°. Scopula occupies entire length of truncature. Serrula very long, extending to outer edge. *Sternum* much longer than wide in ratio 57 : 35, widest in posterior third; slightly produced and rounded posteriorly in front of fourth coxæ which are contiguous. Anteriorly narrowed to less than width of lip, emarginate. Color of sternum dark brown. Surface clothed with long white hair dark at base. Second coxæ the shortest, first coxæ widest as compared with their length, fourth coxæ longest. First coxæ dark brown, the others yellow with brown sides. *Legs* 4123.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.911	2.132	2.132	1.640	1.025	9.840
II	2.460	1.763	1.845	1.435	0.943	8.446
III	2.501	1.476	1.722	1.722	0.943	8.364
IV	3.157	1.640	2.870	2.255	0.984	10.906
Palp	1.230	0.533	0.779	1.025		3.567

First leg heaviest. Femur in middorsal line in the shape of a perfect arc the center of which lies almost on, in reality a little

below the ventral surface of the segment. The sides of the first femur are flat. Tibia is round. Metatarsus is flat below. Both the first tibia and metatarsus are gradually thinner toward the end. Second leg of the same type as the first, but not as thick. Third and fourth legs more slender, their segments, except metatarsi, cylindrical. Legs clothed with white and dark hair and bristles. Trichobothria in two rows on all tarsi, in one row on metatarsi and tibiæ. Claws curved, strong, with a single tooth. Palpal claw wanting. Ungual tufts strongly developed. Hair of white color, flattened and short enough to resemble scales is present in various places on the legs, especially at the end of the fourth metatarsi where it forms a regular white tuft.

Spines. First leg. Femur—dorsal 1-1-1, the first in middle; prolateral 1-2, elsewhere 0. Patella—dorsal 1 bristle at end, elsewhere 0. Tibia—ventral 2 rudimentary, toothlike spines, elsewhere 0. Metatarsus ventral 2-2 rudimentary, toothlike spines, first pair in middle, elsewhere 0. *Second leg* same as first, except: Tibia—ventral 0. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1-1 on right leg, 1-2 on left leg; elsewhere 0. Patella—dorsal 1 bristle at end. Tibia—dorsal 0, prolateral 0-1, retrolateral 1-1, ventral 1p-2. Metatarsus—dorsal 0, prolateral 2 at end, retrolateral 2 at end, ventral 2-2, first pair in middle. *Fourth leg* same as third, except: Femur—prolateral 0. Tibia—prolateral 0, retrolateral 1-1-1, ventral 1p-1p-2. Metatarsus—prolateral 1-1, retrolateral 0-1, ventral 1-2. *Palp.* Femur—dorsal 2 at end, elsewhere only white long hair on sides and still longer black bristles above. A brush of thick hair on inside of terminal joint. *Abdomen* elongate, 9.120 mm. long, 4.800 mm. wide in middle. Back clothed with short black hair, long black hair and yellow lanceolate hair. The latter occupies the median longitudinal band with its ramifications, but is present also on isolated islands on the sides of the abdomen. Anal tubercle with a fringe of long yellow hair. Posterior spinnerets with a longitudinal band of lanceolate yellow hair on inside. Two parallel bald, white lines extend from epigynum almost to spinnerets. A pair of large round spots clothed with lanceolate hair on sides of abdomen a little in front of spinnerets. Anterior spinnerets stouter and shorter than the posterior pair, cone-shaped, dark brown; median and posterior

spinnerets cylindrical. In place of colulus an area clothed with hair. Epigynum large, flat, with a deep median posterior notch and an evenly curved anterior end. The lungs are almost in contact with the epigynum. Habitat: Cerro Iglesia, Brazil, Trinidad.

Freya bifurcata (F. Cambridge) (Figures 141 and 142).

Male. Total with chelicerae 5.687 mm. Carapace 2.788 mm. long, 2.050 mm. wide, 1.353 mm. high. Head flat. Thoracic groove longitudinal, its anterior end touching imaginary line

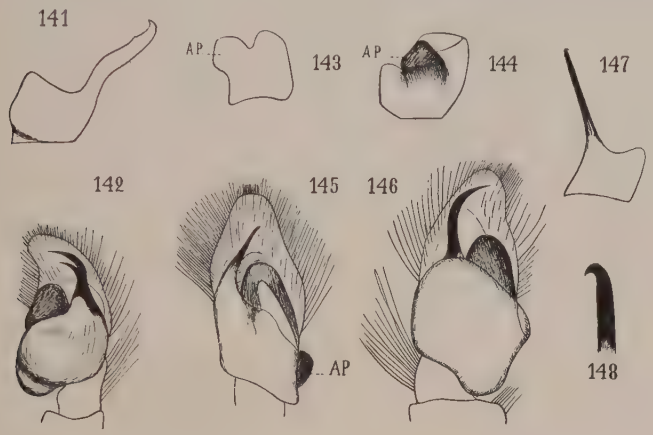


FIGURE 141, *Freya bifurcata* (F. Cambridge), tibia of right palp from above. FIGURE 142, Same, right palp. FIGURE 143, **Freya distincta** sp. nov., tibia of left palp from above. FIGURE 144, Same, tibia of left palp from outside. FIGURE 145, Same, left palp from below. FIGURE 146, *Freya longispina* (F. Cambridge), left palp from below. FIGURE 147, Same, tibia of left palp from outside. FIGURE 148, Same, end of apophysis of the tibia of the palp.

tangent to posterior margins of PLE. Behind eyegroup carapace slopes at first gently, then suddenly forms a steep declivity. Eyegroup much shorter than thoracic portion, and only 1.295 mm. long. First row of eyes 1.776 mm. wide, second row 1.554 mm., third row 1.776 mm. PME nearer PLE than ALE in ratio 8.5:12. Ratio of eyes AME:ALE:PME:PLE = 14:9:2:7. AME subcontiguous, with scales between them, a little more separated from ALE. Viewed from in front, first row of eyes is straight by upper margins. Clypeus high, equal to the radius of

AME and except for a triangle between AME completely covered with white hair which lies parallel to the edge and the ends toward the middorsal line. The triangle between the AME is clothed with rust-brown scalelike hair. AME and ALE surrounded by similar hair. A transverse band of white hair extends from one ALE to the other. A median longitudinal white band extends from the end of the head backwards over two-thirds of the thoracic region. A lateral band of white scale-like hair begins on each side at the clypeus and extends to the posterior edge of the carapace, without, however, merging with its counterpart posteriorly. The margin itself is covered with shiny black hair forming a narrow line. Head, except for portions clothed with white hair, is shiny black with greenish-purple iridescence and has black hair. Rest of carapace behind eyes and between the two lateral white bands and median white band, rust-brown with black hair. Three bristles in a triangle on clypeus between AME; several bristles back of each eye of the first row; a row of three bristles to the outside of the eyegroup on each side; 2 bristles back of each PLE and a transverse row of 6 bristles between the two PLE. *Cheliceræ* comparatively small, parallel, clothed in front with long white hair. Basal joint 0.888 mm. long. Margins almost transverse. Promargin with 2 strong, sharp teeth, the distal one of which is the strongest. Retromargin with a single sharp tooth. Scopulæ wanting and hair on margins scanty. Fang normal. *Lip* longer than wide in ratio 13:11, gradually narrowed toward end and rounded, articulated to maxillæ one third from base, reaching beyond their middle. *Maxillæ* with short truncature, rounded end and sharply produced outer angle, so that outer margin is strongly concave. Scopula dense, light greyish-brown. Serrula reaching to base of outer angle. *Sternum* moderately convex, longer than wide in ratio 30:20, oval, truncated in front to width of lip. First coxæ wide apart, fourth coxæ contiguous. Sternum clothed with grey and white erect hair. Legs about the same size. Spines long, strong and numerous. Hair on legs white and black. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts well developed. Claws slightly dissimilar. Proclaw with 2 teeth followed by minute tubercles, retroclaw with 4 teeth. *Legs* 3142.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.628	0.999	1.295	1.073	0.703	5.698
II	1.480	0.851	0.851	0.888	0.592	4.662
III	1.850	0.888	1.036	1.258	0.666	5.698
IV	1.739	0.777	1.073	1.258	0.592	5.439
Palp	0.777	0.370	0.333	0.888		2.368

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, retrolateral 1-1, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1 bristles, prolateral 1-1-1, retrolateral 0, ventral 2-2-2, first pair near base. Metatarsus—dorsal 0, prolateral 1 small, at end; retrolateral 0, ventral 2-2. *Second leg* as first, except: Femur—retrolateral 1-2. Tibia—ventral 1r-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-2, retrolateral 1-2, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, the second a bristle, prolateral 1v-1d-1v-1d, retrolateral 1-1-1, ventral 1p-2. Metatarsus—dorsal 0, prolateral 1-2, retrolateral 1-1d-2, ventral 2-2. *Fourth leg* same as third, except: Femur—prolateral 1-1. *Palp.* Patella—1-1 dorsal black bristles, elsewhere 0. Femur and patella of palp thickly clothed with white hair on dorsal surface. *Abdomen* black above with a median, longitudinal white band which is as wide as the spinnerets. Black area bordered laterally and anteriorly by a white band. In front of the anterior white band abdomen again black. Venter dark grey. Sternum and legs yellow except first patella, tibia and metatarsus. Cheliceræ dark brown. Lip and maxillæ dark brown with light yellow ends. Femur of palp dark brown below, patella and tibia yellow below, bulb almost black. End of embolus deeply cleft, the two teeth of the fork of different length. Tibial apophysis long, straight, slightly notched at end. Habitat: Panama.

Freya distincta sp. nov. (Figures 143-145).

Male. Total with cheliceræ 7.68 mm. Carapace 3.500 mm. long, 2.625 mm. wide, 1.925 mm. high. Head almost flat. Thoracic groove longitudinal, its anterior end considerably in advance of the imaginary transverse line tangent to posterior edges of PLE, so that posterior edge of head is in consequence deeply emarginate in middle. Eyegroup 2.091 mm. long, thoracic portion 2.870 mm. long. First row of eyes 2.460 mm. wide, second row 2.050 mm., third row 2.337 mm. Ratio of eyes AME:

ALE : PME : PLE = 21 : 13 : 2.5 : 12. AME subcontiguous, but distinctly separated from ALE. PME nearer PLE than ALE in ratio 10.5 : 13, and are separated from PLE by less than the diameter of the latter. Clypeus equal to half the radius of AME, clothed with erect, white hair. Three bristles in a triangle on clypeus between AME and two bristles on each side in front of AME. A row of bristles on each side to the outside of the eyegroup. Two bristles back of each PLE. Several, apparently irregularly arranged, bristles on head. Short, brown hair encircles each anterior eye. Head dark, clothed with recumbent brown hair which is directed forward. Posterior declivity red-brown with black hair. Sides of carapace clothed with white hair directed upward. Margins black. *Chelicerœ* parallel, stout, clothed in front with long white hair which is so thick that together with the white hair of the clypeus they conceal the base of the cheliceræ. Margins very slightly oblique. Promargin with 2 teeth of which the distal one is largest. Retromargin with a single, sharp but not very strong tooth. A long white not very thick scopula on promargin, and a row of hair on retromargin. Fang rather long, evenly curved. *Lip* longer than wide in ratio 16 : 13, gradually but considerably narrowed toward end where it is truncated, reaching to middle of maxillæ. *Maxillœ* greatly dilated in front. Truncature short, clothed with a brown scopula. Anterior edge curved. Outer angle produced, sharp. Lateral edge concave. Serrula reaching to outer angle. *Sternum* longer than wide in ratio 44 : 26, oval, truncated anteriorly to more than width of lip. First coxæ wide apart, fourth coxæ contiguous. Sternum moderately convex, clothed with short hair. First leg heavier than the others. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts very thick. Claws dissimilar. Proclaw with a series of closely set teeth. Retroclaw with but 2 teeth. *Legs* 4132.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.183	1.258	1.850	1.443	0.830	7.534
II	1.998	1.258	1.443	1.184	0.740	6.624
III	2.294	1.110	1.517	1.776	0.777	7.474
IV	2.479	1.110	1.813	1.998	0.888	8.288
Palp	1.184	0.555	0.481	1.036		3.256

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end,

elsewhere 0. Patella—dorsal 1-1, elsewhere 0. Tibia—dorsal 0, prolateral 1-0-1, retrolateral 0, ventral 2-2-2, first pair near base. Metatarsus—ventral 2-2, elsewhere 0. *Second leg* same as first, except: Patella—prolateral 1. Tibia—ventral 1r-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-2, retrolateral 0-1, ventral 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1 bristles, prolateral 1-1d-1, retrolateral 1-1-1, ventral 1p-2. Metatarsus—dorsal 0, prolateral 1-0-2, retrolateral 1-1-2, ventral 2-2. *Fourth leg* same as third, except: Femur—prolateral 0-1. *Palp.* Femur—dorsal 1-1-1, elsewhere 0. Patella—dorsal 1-1-1 bristles, elsewhere 0. Tibia—dorsal several bristles, elsewhere 0. Tibia is bilobed at end, tibial apophysis short, blunt, rounded. Embolus arises on proside of bulb. Bulb produced at base. Anterior legs dark brown with yellow tarsi. Posterior legs mottled with yellow. Sternum yellow. Abdomen clothed with long black and short rust-brown hair on the dorsal surface, with only short black hair below. Pattern indistinct. Small yellow spots, a few transverse lines above and a transverse yellow band below in front of the spinnerets. Colulus replaced by a tuft of long hair. Spinnerets cylindrical, posterior pair considerably longer and more slender than anterior pair. Habitat: Wilcox camp on San Lorenzo River.

Freya longispina (F. Cambridge) (Figures 146-148).

Male. Total with cheliceræ 7.2 mm. Carapace 3.485 mm. long, 2.583 mm. wide, 1.640 mm. high. First row of eyes 2.132 mm. wide, second 1.927 mm., third row 2.091 mm. PME nearer PLE than ALE in ratio 10:13. Ratio of eyes AME:ALE:PME:PLE = 18:10:2.75:9. Viewed from in front, first row of eyes is straight by upper margins. AME sub-contiguous, but clearly separated by scales, and still more separated from ALE. Clypeus equal to 8/18 of the diameter of AME. Mouthparts as in *F. bifurcata,* but outer angle of maxillæ much less produced. It is altogether a larger species than *F. bifurcata,* though less conspicuous and not as brilliantly colored. The spines are long, light yellowish-brown. Legs more or less alike, clothed with white and black hair. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts well developed. Claws slightly dissimilar. Proclaw with 2 teeth followed proximately by minute tubercle. Retroclaw with 4 teeth. *Legs* 3142.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.924	1.332	1.628	1.332	0.814	7.030
II	1.850	1.036	1.184	0.962	0.518	5.550
III	2.332	1.184	1.295	1.554	0.777	7.142
IV	2.109	0.888	1.369	1.480	0.740	6.590
Palp	0.999	0.518	0.407	1.221		3.145

Spines. First leg. Femur—dorsal 1-1-1, prolateral 0-2, retrolateral 1-1, ventral 0. Patella—dorsal 1-1-1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1-1 bristles, prolateral 1-1d-1, retrolateral 0, ventral 2-2-2, first pair near base. Metatarsus—prolateral 1 at end, ventral 2-2, elsewhere 0. *Second leg* same as first, except: Femur—retrolateral 1-2. Tibia—ventral 1r-2-2. Metatarsus—prolateral 1-1, retrolateral 0-1. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-2, retrolateral 1-2, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1-1, the first a spine, the second and third bristles, prolateral 1v-1d-1v-1d, retrolateral 1v-1d-1v-1d, ventral 1p-2. Metatarsus—dorsal 0, prolateral 1-2, retrolateral 1-1d-2, ventral 2-2. *Fourth leg* same as third, except: Femur—retrolateral 2 at end. Metatarsus—prolateral 1-1-2. *Palp.* Femur—dorsal 0-1. Patella—dorsal 1-1 bristles. Tibia—dorsal 0-1 bristle, prolateral several bristles, elsewhere 0. Posterior spinnerets the longest. Colulus replaced by a circle of hair. Habitat: Guatemala and Panama.

Phiale delecta (Peckham) (Figure 149).

Female. Total with chelicerae 5.33 mm. Carapace 2.460 mm. long, 1.722 mm. wide, 1.025 mm. high. Head flat. Thoracic groove longitudinal, small; its anterior end touches the imaginary transverse line tangent to posterior edges of PLE. Eyegroup 1.369 mm. long, thoracic portion 1.480 mm. long. First row of eyes 1.369 mm. wide, second row 1.221 mm., third row 1.443 mm. PME nearer ALE than PLE in ratio 7:8, separated from PLE by more than the diameter of the latter. Ratio of eyes AME: ALE:PME:PLE = 13:6:2:6. AME separated from each other and from ALE only by the width of a scale of the type which completely encircle the eyes, *i. e.* practically contiguous. Clypeus equal to half the radius of AME, clothed with white scales. One white bristle on edge of clypeus above the chelicera of each side, 3 bristles on each side in front of AME. Three dark

bristles in a triangle on clypeus between AME. (In all 8 white and 3 dark bristles.) Head clothed with rather numerous black bristles of varying length and also with recumbent white hair directed forward. Eyegroup bordered laterally by a band of rust-brown hair. Posterior declivity with white hair in middle and black hair on sides. Sides of carapace with white hair and with a few black hairs on margin. *Chelicerœ* weak, dark yellow, devoid of hair. Promargin with 2 small sharp teeth of which the distal one is largest. Retromargin with a single, small, sharp tooth. Scanty hair on both margins. Fang evenly curved. *Lip* yellow, as long as wide, slightly narrowed and rounded in front. *Maxillæ* yellow, rounded in front, with outer edge straight. Scopula thick. Serrula occupying the edge, extending considerably toward base of maxilla. *Sternum* yellow, flat, oval, longer than wide in ratio 25 : 17, considerably narrowed in front. First coxæ separated by more than width of lip, but are closer together than third coxæ. Fourth coxæ contiguous. All coxæ and trochanters yellow. Sternum clothed with white hair, legs with black hair. Trichobothria in two rows on all tibiæ, in one row on all metatarsi and tarsi. Ungual tufts grey, well developed. Claws with 4 or 5 teeth. *Legs* 4312.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.110	0.666	0.740	0.555	0.481	3.552
II	0.999	0.720	0.629	0.518	0.407	3.273
III	1.332	0.666	0.777	0.851	0.518	4.144
IV	1.332	0.740	0.888	0.999	0.481	4.444

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, elsewhere 0. Patella—dorsal 1-1 bristles, elsewhere 0. Tibia—dorsal 0-1 bristle, prolateral 1-1, retrolateral 0, ventral 2-2-2, first pair near base. Metatarsus—ventral 2-2, elsewhere 0. *Second leg* same as first, except: Tibia—ventral 1r-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-2, retrolateral 0-1, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1, the first a spine, the second a bristle; prolateral 1-1-1, retrolateral 1-1-1, ventral 1p-2. Metatarsus—dorsal 0, prolateral 1-2, retrolateral 1-1-2, ventral 2-2. *Fourth leg* same as third, except: Femur—prolateral 0-1. Metatarsus—prolateral 1-1-2, ventral 1p-2-2. Palpal claw wanting. Epigynum flat, with two round openings in front, leading into two round receptacles which are visible through the skin and are

situated behind the openings. A median oval perforation near edge. Colulus represented by a triangular sclerite covered with hair. Spinnerets about equal in length. Venter yellow, clothed with white hair. Sides of abdomen striped longitudinally, the

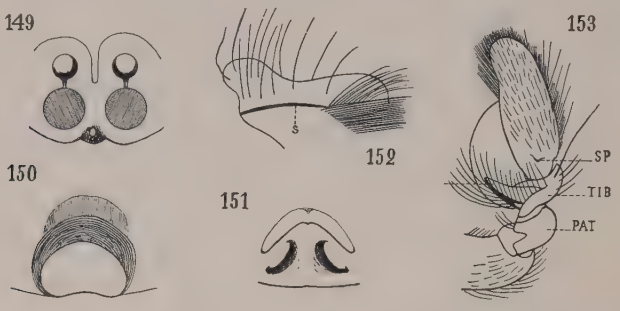


FIGURE 149, *Phiale delecta* (Peckham), epigynum. FIGURE 150, *Phiale simplicicava* (F. Cambridge), epigynum. FIGURE 151, *Phiale bicuspidata* (F. Cambridge), epigynum. FIGURE 152, *Menemerus bivittatus* (Dufour), end of left maxilla viewed obliquely from above. FIGURE 153, Same, left palp from outside.

dark stripes formed by dark hair, the light stripes by white hair. Dorsum with four transverse recurved stripes in anterior half. First stripe black, second and fourth yellowish-white, third red. The third is the widest stripe. Posterior half of dorsum pitch-black with a median row of four yellowish-white small spots diminishing in size posteriorly, and two pairs of round, snow-white spots. Anterior spinnerets yellow, posterior spinnerets brown. Legs yellow. Habitat: Panama, Costa Rica, Guatemala and Mexico.

Phiale simplicicava (F. Cambridge) (Figure 150).

Female. Total with cheliceræ 8.16 mm. Carapace 4.100 mm. long, 2.952 mm. wide, 1.886 mm. high. Head flat, black; its posterior edge emarginate. Thoracic groove longitudinal, its anterior end touching the imaginary line tangent to posterior edges of PLE. Eyegroup 1.845 mm. long, thoracic portion 2.114 mm. long. First row of eyes 2.296 mm. wide, second row 2.050 mm., third row 2.337 mm. PME nearer PLE than ALE in ratio 11:15, separated from PLE by more than the diameter of the latter. Ratio of eyes AME:ALE:PME:PLE = 19:11:3:9. AME are distinctly separated from each other and still

more so from ALE. Clypeus equal to 1/3 the radius of AME, fringed with long, white hair. Three bristles in a triangle on clypeus between AME, and two white bristles on each side in front of AME. Numerous bristles of varying size on head irregularly arranged. White, recumbent, iridescent hair also present on head. Thoracic portion red-brown with recumbent white hair. Margin black and slightly rebordered. *Chelicerœ* stout and strong, dark brown. Promargin with two teeth of which the distal one is the largest. Retromargin with a single strong, sharp tooth. A regular yellow scopula on promargin, but only scattered hair on retromargin. *Lip* light brown, longer than wide in ratio 18:14, articulated to maxillæ one-third from base, gradually narrowed in front and truncated. It reaches beyond middle of maxillæ. *Maxillæ* light brown with white truncature, widened and rounded in front. Scopula brown. Serrula marginal, not reaching to middle of outer angle. *Sternum* yellow, clothed with white hair; oval, longer than wide in ratio 44:27, narrowed in front to less than width of lip. First coxæ separated from each other by more than width of lip, but less than coxæ of third pair. Fourth coxæ contiguous. All coxæ, trochanters and ventral surface of femora yellow. Legs clothed with white and dark hair. Trichobothria in two rows on all tibiæ; in one row on metatarsi and tarsi. Ungual tufts well developed. Claws dissimilar. Proclaw with a series of closely set teeth, retroclaw with 4 teeth. *Legs* 3412.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.050	1.353	1.476	1.189	0.738	6.806
II	1.845	1.230	1.230	0.984	0.738	6.027
III	2.542	1.353	1.353	1.640	0.902	7.790
IV	2.460	1.189	1.435	1.850	0.818	7.752
Palp	0.962	0.592	0.481	0.925		2.960

Spines. First leg. Femur—dorsal 1-1-1, prolateral 0-2, retrolateral 1-1, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, elsewhere 0. Tibia—dorsal 1-1 bristles, prolateral 1-1, retrolateral 0, ventral 2-2-2, first pair near base. Metatarsus—ventral 2-2, elsewhere 0. *Second leg* same as first, except: Femur—retrolateral 1-2. Tibia—ventral 1r-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-2, retrolateral 0-2, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia dorsal 1-1 bristles, prolateral 1-1-1, retrolateral 1-1-1, ventral

1p-2. Metatarsus—dorsal 0, prolateral 1-0-2, retrolateral 1-1-2, ventral 2-2. *Fourth leg* same as third, except: Femur prolateral 0-2. Metatarsus—prolateral 1-1-2. *Palp.* Femur—dorsal 1. Patella—dorsal 1-1 bristles. Tibia—dorsal 1-1 bristles, elsewhere 0. Many long white hairs on tibia of palp. *Abdomen* clothed with brown and yellow hair above. Venter light yellow. Spinnerets of the usual type, but median pair much shorter than the others. Epigynum dark brown. Habitat: Panama and Mexico.

Phiale bicuspidata (F. Cambridge) (Figure 151).

Female. Total with cheliceræ 6.75 mm. Carapace 3.321 mm. long, 2.296 mm. wide, 1.558 mm. high. Head flat, its posterior line strongly emarginate. Thoracic groove longitudinal, its anterior end touching the imaginary transverse line tangent to posterior edges of PLE. Eyegroup 1.845 mm. long, thoracic portion 1.845 mm. long. First row of eyes 2.173 mm. wide, second row 1.927 mm., third row 2.132 mm. PME nearer PLE than ALE in ratio 10:15, separated from PLE by the diameter of the latter. Ratio of eyes AME:ALE:PME:PLE = 18:11:2.5:10. AME distinctly separated from each other and more so from ALE. Clypeus 1/3 of the diameter of AME, fringed with long white hair. Each eye of the first row encircled by white hair. Viewed from in front, first row of eyes is straight by upper margins. Three bristles in a triangle on clypeus between AME and two bristles on each side in front of AME. Head with many bristles of variable size, but hair on head wanting. Sides of carapace with white and brown hair. *Chelicerœ* stout, reddish with green iridescence. Margins oblique. Promargin with 2 teeth of which the distal one is much the larger. Retromargin with a single tooth which is strong and pointed. Hair on margins scanty. *Lip* longer than wide in ratio 14:10, gradually narrowed in front and rounded. *Maxillæ* rounded in front, with thick scopula and straight outer edge. Serrula marginal but not very long. *Sternum* oval, longer than wide in ratio 35:23, clothed with fine, erect hair. First coxæ closer together than third pair, but wider apart than lip. Fourth coxæ contiguous. Legs clothed with black and white hair. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts well developed. Claws slightly dissimilar. Proclaw with

8 closely set teeth. Retroclaw with 6 teeth increasing in length distally, the first tooth being quite small. *Legs* 4312.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.739	1.184	1.184	0.888	0.666	5.661
II	1.665	1.036	1.036	0.851	0.592	5.189
III	2.025	1.036	1.147	1.295	0.703	6.206
IV	1.999	0.925	1.406	1.591	0.777	6.698
Palp	0.851	0.407	0.444	0.666		2.368

Spines. First leg. Femur—dorsal 1-1-1, prolateral 0-2, elsewhere 0. Patella—dorsal 1-1 bristles, elsewhere 0. Tibia—dorsal 1-1 bristles, prolateral 1-1, retrolateral 0, ventral 2-2-2, first pair near base. Metatarsus—ventral 2-2, elsewhere 0. *Second leg* same as first, except: Femur—retrolateral 0-1. Patella—prolateral 1. Tibia—ventral 1r-2-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-2, retrolateral 0-1, ventral 0. Patella—dorsal 1-1 bristles, prolateral 1, retrolateral 1, ventral 0. Tibia—dorsal 1-1 bristles, prolateral 1-1-1, retrolateral 1-1-1, ventral 1p-2. Metatarsus—dorsal 0, prolateral 1-2, retrolateral 1-2, ventral 2-2. *Fourth leg* same as third, except: Femur—prolateral 0, retrolateral 0-1. Metatarsus—prolateral 1-1d-2, retrolateral 1-1-2, ventral 1p-2. *Palp.* Femur—dorsal 1. Patella—dorsal 1-1 bristles. Tibia—dorsal 1-1-1 bristles, elsewhere 0. Coloration as described by F. Cambridge. Abdomen clothed above with black, erect hair and red, recumbent hair. Habitat: Costa Rica and Panama.

Menemerus bivittatus (Dufour) (Figures 152 and 153).

This cosmopolitan species has been described and figured many times, but careful measurements and detailed description of the arrangement of spines are lacking. The male described below corresponds closely to the variety described by F. Cambridge as *Marpissa melanognatha* Lucas.

Male. Total 5.35 mm. Carapace 2.665 mm. long, 2.050 mm. wide; flat, with depression behind eyegroup, containing longitudinal thoracic groove. Carapace considerably wider than posterior row of eyes. Eyegroup 1.406 mm. long, thoracic portion 1.628 mm. long. First row of eyes 1.628 mm. wide, second row 1.480 mm., third row 1.628 mm. PME nearer PLE than ALE in ratio 8:12, separated from PLE by 8/7 of the diameter of the latter. Ratio of eyes AME:ALE:PME:PLE = 13:8:1.75:7.

AME contiguous but slightly separated from ALE. Viewed from in front, first row of eyes is straight by upper margins. Clypeus low, clothed with white hair which is directed obliquely inward and downward. One bristle on clypeus between AME. A semicircle of bristles behind each anterior eye, a row of four bristles on each side between ALE and PLE to the outside of the eyegroup, 1 bristle back of each PLE and several bristles on head which is black with purple iridescence. Elsewhere carapace reddish-brown with a lateral marginal band of white hair, with brown hair around each eye, with black hair and bristles on the sides of posterior declivity and with a few white hairs around the thoracic groove and on head. *Cheliceræ* with transversely corrugated surface clothed at base with white hair, elsewhere with black hair. Promargin with 2 teeth, retromargin with a single sharp tooth. A weak scopula on promargin and a row of hairs on retromargin. Fang normal, not sinuous, but beginning about 1/3 from base rather slender. *Lip* longer than wide in ratio 17 : 12, considerably narrowed toward end, articulated to maxillæ in middle. *Maxillæ* converging, with small truncature, evenly rounded at end, but with a sharply projecting angle in middle of outer margin, visible only if first coxæ have been removed. The lower surface of maxillæ is peculiarly concave, while the upper one is quite flat. *Sternum* flat, longer than wide in ratio 32 : 20, narrower in front than behind, trucated in front to less than width of lip. First coxæ close together, fourth coxæ contiguous. First pair of legs heaviest, but the difference not striking. Spines on femora long, curved, other spines short, normal. Legs clothed with three types of hair: black, white and orange, the latter recumbent. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts well developed. Claws dissimilar. Proclaw of first leg with a long series of fine teeth, retroclaw smooth. Proclaw of fourth leg with a series of teeth, retroclaw with 2 small teeth. *Legs* 4123.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.665	1.036	1.221	0.999	0.518	5.439
II	1.554	0.962	0.999	0.851	0.518	4.884
III	1.406	0.777	0.925	0.999	0.555	4.662
IV	1.813	0.888	1.406	1.221	0.629	5.957
Palp	0.888	0.296	0.222	0.851		2.257

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, elsewhere 0. Patella—1-1 bristles, elsewhere 0. Tibia—dorsal 1 bristle, ventral 2-2-2, first pair near base, elsewhere 0. Metatarsus —dorsal 1-1-1 bristles, ventral 2-2, elsewhere 0. *Second leg* same as first, except: Femur—prolateral 1 at end. Tibia—ventral 1r-0-1p. *Third leg.* Femur—dorsal 1-1-1, prolateral 1-1, retrolateral 0-1, ventral 0. Patella—0. Tibia—dorsal 0, prolateral 0-1, retrolateral 0-1, ventral 0-2. Metatarsus—dorsal 0, prolateral 0-2, retrolateral 0-2, ventral 0-2. *Fourth leg* same as third, except: Femur—prolateral 0-1. Tibia—prolateral 0. Metatarsus—prolateral 0-1. *Palp* without spines. Bristles on palp may be recognized by their black color, otherwise similar to the white hair by which they are concealed and which is as long and as stiff as the bristles. Femur of palp curved downward and flattened on prolateral surface. Patella short. Tibia shorter than patella and so distorted that at first glance the terminal joint appears to be articulated not in line with the preceding joints, but considerably to the inside. Nevertheless the articulation is normal and the peculiar appearance is due to the fact that the tibial apophysis is closely applied to the base of the terminal joint. Prolateral surface of the tibia is clothed with long white hair, while the retrosurface is fringed with a row of black hair almost entirely concealing the tibial apophysis. Tibia of palp shorter than wide. Terminal joint many times longer than tibia, with a short blunt retrodorsal spur at base. The base of the bulb reaches to base of tibia. Embolus short, with a broad base. Spinnerets equal in length, median and posterior pair cylindrical, anterior pair much stouter, cone-shaped. Colulus replaced by a few hairs. Abdomen light yellow below, clothed with simple hair. Abdomen above buff with a longitudinal median band in anterior half, clothed with dark and orange hair and with long white hair on shoulders.

Dendryphantes (*Metaphidippus*) *lanceolatus* (F. Cambridge) (Figure 154).

Male. Total with cheliceræ 6.7 mm. Carapace 2.500 mm. long, 2.296 mm. wide, 1.295 mm. high. Thoracic groove longitudinal, very small, situated in a shallow depression considerably behind the imaginary transverse line tangent to posterior margins of PLE. Head flat. First row of eyes 1.628 mm. long, second row

1.498 mm., third row 1.739 mm. Eyegroup 1.369 mm. long, thoracic portion 1.739 mm. long. Viewed from in front first row of eyes straight by upper margins. PME nearer ALE than PLE in ratio 9:11, separated from PLE by 11/6.5 of the diameter of the latter. Ratio of eyes AME:ALE:PME:PLE = 13:7:1.5:6.5. AME distinctly separated from each other and still more so from ALE. Clypeus equal to 3/13 of the diameter

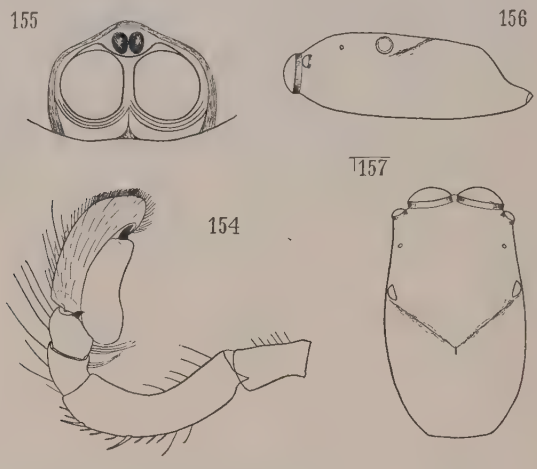


FIGURE 154, *Dendryphantes* (*Metaphidippus*) *lanceolatus* (F. Cambridge), right palp. FIGURE 155, **Mica longithorax** gen. et sp. nov., epigynum. FIGURE 156, Same, side view of carapace. FIGURE 157, Same, carapace from above.

of AME. 1-1 median bristles on clypeus and a transverse row of 3 bristles on each side in front of AME. 3 small bristles on each side between AME and ALE, several bristles on each side to the outside of the eyegroup, a transverse row of 4 equidistant bristles behind the third row of eyes, a transverse row of 6 bristles behind the thoracic groove and several irregularly placed bristles farther back. Sides of carapace with a white band of scales, beginning immediately under the eyes, widest under PLE, where it occupies almost half of the carapace, then becoming quite attenuated behind ALE. AME situated on quite short, dark brown cylindrical bases. ALE and PME on each side of a joint black field. PLE on each side on a round black field of its own. *Chelicerœ* powerful, strongly divergent. Basal joint 1.258 mm. long. Margins long,

oblique. Promargin with 2 contiguous teeth of which the proximal is quite small, the distal long, but blunt. Retromargin with a single, powerful, pointed tooth. A few hairs on both margins, but no real scopula. Fang short, evenly curved. *Lip* longer than wide in ratio 17: 13, considerably narrowed in front and truncated, articulated to maxillæ before middle. *Maxillæ* inclined over lip, truncatures strongly divergent. Scopula very thick and even, except for the tuft of long hair at upper end. Outer angle sharp. Anterior edge straight, lateral edge almost straight. Serrula straight, extending to outer angle. *Sternum* flat, oval, longer than wide in ratio 30:22, truncated in front to width of lip. First coxæ wider apart than width of lip, fourth coxæ contiguous. First pair of legs much heavier than the others. Femur strongly convex dorsally, flattened prolaterally and ventrally in distal half. Tibia cylindrical. Metatarsus and tarsus much more slender than tibia. Tarsus stouter at end than at base. Spines short and stout, except the dorsal spines on femora, which are longer, more slender and curved. Trichobothria in two rows on all tibiæ, in one row on metatarsi and tarsi. Ungual tufts thick. Claws dissimilar. Proclaw with a long series of equal and closely set teeth, retroclaw with only 3 teeth. *Legs* 1423.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.850	1.221	1.369	1.110	0.555	6.105
II	1.443	0.888	0.888	0.814	0.481	4.514
III	1.443	0.703	0.777	0.814	0.555	4.292
IV	1.628	0.851	0.999	0.999	0.555	5.032
Palp	0.814	0.310	0.222	0.740		2.086

Spines. First leg. Femur—dorsal 1-1-1, prolateral 2 at end, elsewhere 0. Patella—dorsal 1-1-1-1 fine bristles, elsewhere 0. Tibia—dorsal 1-1 bristles, ventral 2-2-2, first proventral spine beyond middle, first retroventral spine a little before middle, elsewhere 0. Metatarsus—ventral 2-2, first pair in middle, elsewhere 0. *Second leg* same as first, except: Femur—retrolateral 0-1. Patella—prolateral 1. Tibia—ventral 1r-1r-2. *Third leg.* Femur—dorsal 1-1-1, prolateral 0-2, retrolateral 0-1, ventral 0. Patella—dorsal 1-1-1-1 bristles, retrolateral 1, elsewhere 0. Tibia—dorsal 1-1-1 bristles, prolateral 0-1, retrolateral 1-1, ventral 0-2, at end. Metatarsus—dorsal 0, prolateral 0-2, retrolateral 0-2, ventral 1p-2. *Fourth leg* same as third, except: Femur—prolateral 0-1. Tibia

—prolateral 0. Metatarsus—prolateral 0-1, retrolateral 0-1. *Palp.* Femur—dorsal 1-1. Patella—dorsal 1, bristle. Tibia—dorsal 1 bristle, prolateral several bristles, elsewhere 0. White scales on all femora and a few on all patellæ. Here and there a few scales also on all other joints. Femur of palp strongly curved downward, with white scales. Patella of palp longer than wide in ratio 9:7. Tibia of palp shorter than patella, shorter than wide. Tibial apophysis very short, toothlike, directed downward. Terminal joint obliquely truncated at end, white. Bulb reaching almost to base of tibia. Embolus short and stout, originating from the end of the bulb. Anal tubercle long, with a fringe of long hair. Spinnerets of the usual type. Colulus wanting. Abdomen sparsely clothed with hair and white scales. A longitudinal band of white scales borders on the median red field on each side and the two fuse together in front. Anterior border of abdomen with a fringe of long bristles which are rather far apart. Color as in type specimen described by F. Cambridge. Habitat: Panama, Costa Rica, Guatemala and Mexico.

Mica gen. nov.

Retromargin of cheliceræ with a single, pointed tooth (divisio Unidentati), promargin also with a single tooth. Carapace low, long and narrow; eyegroup much shorter than thoracic portion, wider than long. First row of eyes shorter than third row and eyes of second row slightly nearer ALE than PLE. Clypeus low. Sternum long and narrow. Legs in order 4132. First tarsi as long as metatarsi, fourth tarsi shorter than metatarsi. Spines completely wanting except a single prolateral apical spine on first metatarsus. Scales present on body and legs. Type **M. longithorax.**

Mica longithorax sp. nov. (Figures 155-157).

Female. Total 2.775 mm. Carapace 1.406 mm. long, 0.777 mm. wide, 0.481 mm. high. Eyegroup 0.555 mm. long, rest of carapace behind eyegroup 0.830 mm. long. Carapace low, sloping down from PME anteriorly and some distance behind PLE posteriorly. This posterior declivity is at first steep, then gentle, so that the median longitudinal line is concave. Distance between PLE and the point at which posterior declivity begins is 0.444 mm.

long, and the posterior declivity is also 0.444 mm. long. Distance between vertex of AME and thoracic groove is 0.888 mm. long. Thoracic groove longitudinal, situated considerably behind PLE. Cephalothoracic sulci pass immediately behind PLE, converge to a point on thoracic groove and form between them an angle of 80°. Carapace widest in region of thoracic groove, wider anteriorly than posteriorly. Viewed from in front, first row of eyes is straight by upper margins, curved upward by centers. First row of eyes 0.666 mm. wide, second row 0.592 mm., third row 0.740 mm. Ratio of eyes AME : ALE : PME : PLE = 6.75 : 2.75 : 1 : 3.25. PME nearer ALE than PLE in ratio 4.5 : 5, separated from PLE by 5/3.25 of the diameter of the latter. Clypeus equal to 2/6.75 of the diameter of AME. White scales between AME and on clypeus. Surface of carapace shiny, minutely punctate, dark chestnut-brown with white scales along the cephalothoracic sulci. *Cheliceræ* short and stout. Promargin with one tooth, retromargin with one pointed tooth. A light scopula on promargin. *Lip* slightly wider than long, articulated to maxillæ in middle, deeply excavate laterally in proximal half, strongly narrowed and rounded in front. Lip does not reach middle of maxillæ. *Maxillæ* with long truncatures which are almost in continuation with inner edge and between them form only a very small angle. Anterior edge almost straight. Outer angle about 90°. *Sternum* more than twice as long as wide in ratio 18 : 8, gradually narrowed to a point behind, truncated in front. First coxæ separated by more than width of lip, fourth coxæ contiguous. *Legs* 4132.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	0.555	0.296	0.370	0.259	0.259	1.739
II	0.444	0.259	0.296	0.222	0.222	1.443
III	0.481	0.240	0.333	0.296	0.259	1.609
IV	0.666	0.296	0.555	0.407	0.296	2.220
Palp	0.296	0.166	0.129	0.222.		0.813

First pair of femora thickened. Spines on legs absent, except a single prolateral apical spine on first metatarsus. There is, however, a long, erect bristle at end of fourth patella and another at end of fourth tibia. Hair on legs also rather sparse. A few random white scales on femora. Three pairs of trichobothria on tibiæ and a row of three or four trichobothria on metatarsi and tarsi. Ungual tufts shorter than claws. Claws dissimilar. Pro-

claw with 8 long, parallel closely set teeth. Retroclaw with but 3 granules in place of teeth. Palpi and first, second and third pair of legs yellow with a black line along prolateral surface. Fourth leg brown except patella and tarsus, which are yellow. All coxæ and trochanters yellow. Abdomen light below, dark above, but so wrinkled owing to poor preservation that neither pattern nor color can be determined definitely. Apparently there is a pair of quite small white spots at anterior end and another pair considerably larger a little beyond middle. These spots are formed by white scales. Epigynum flat and very large. Habitat: Trijoles, Canal Zone.

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 42, No. 3, August, 1925

ON TISSUE CULTURES OF THE PLACENTA OF THE RABBIT [1]

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THREE PLATES (TWENTY-ONE FIGURES)

The tissue of the placenta has not been cultivated in vitro up to the present time. Its behavior outside the body would be of great interest, and it would be especially important to know whether the trophoblastic syncytium is capable of independent existence and growth in vitro.

Regarding the literature of the question there is little to say, since it is limited to the short article of Maximow, read before the Meeting of the American Association of Anatomists in Chicago in 1923. Explanting very young rabbit blastoderms in the stage of the primitive streak, he saw the primordium of the ectoplacenta, which surrounds the embryonic area, produce a large number of proliferating trophoblastic elements. They hypertrophied and fused together, forming huge multinuclear ameboid giant cells, which penetrated into the tissues of the growing embryo and destroyed them. However, the experiments of Maximow were made with very young stages of pregnancy, where only the first primordium of the trophoblast is present.

My aim was to test with the method of tissue culture the placenta of later stages, where large quantities of a ready trophoblastic syncytium are present and where the organ is fully developed and displays intense functional activity.

MATERIAL AND METHODS

Rabbits were used for my experiments, because we possess a very detailed knowledge of the development and structure

[1] From the Osborn Zoölogical Laboratory, Yale University.

of the placenta of this animal and because the blood of the rabbit clots slowly; this facilitates the preparation of the necessary nutritive medium. The stages of pregnancy used were from fifteen to eighteen days. Tissue fragments, taken from the most superficial parts of the ectoplacenta, were explanted into pure plasma or into plasma mixed with various tissue extracts. For obtaining the nutritive medium, blood, drawn directly from the heart by puncture with a syringe or through a cannula from the carotid into cooled paraffined containers, was centrifuged. The plasma was diluted with Ringer solution in the ratio of two to one.

Extracts of embryos, bone marrow, and ovary were used. The tissues were usually taken from the mother of the embryos and were prepared by grinding in a mortar with sand or pulverized glass, followed by diluting the pulp with Ringer solution and by centrifuging.

For facilitating the transplantation of the cultures into new medium, i.e., the so-called subculturing, I used the method of Maximow. This investigator places the drop of plasma with the explant not directly on the surface of the large square cover-slip, covering the hollow slide, but on the surface of a smaller round cover-slip, fastened to the surface of the larger square by means of a small drop of Ringer's solution. After the preparation of the culture, the slide has to be turned over, so that the plasma drop with the growing tissue becomes transformed from a hanging drop into a lying one. The latter condition is important, because the fibrin is very easily liquefied and the hanging drop in such a case at once runs off from the tissue.

The living cultures were studied at a temperature of 37° and photographed. However, as the layer of the tissue is always relatively thick and not sufficiently transparent, fixed and stained preparations are absolutely necessary for the elucidation of the transformations undergone by the various tissues. For fixation Zenker-formol, Zenker-formol-osmic acid and Champy's solution were used. The fixed cultures were removed from the cover-slips by means of a safety-

razor blade; they were embedded in celloidin and cut parallel to the surface in serial sections of 8 μ thickness. For staining I used, after Zenker-formol, eosin-azure or hematoxylin-eosin, after the liquids containing osmic acids, iron-hematoxylin, sometimes combined with Altmann's acid fuchsin stain.

ON THE NORMAL STRUCTURE OF THE RABBIT'S PLACENTA

A detailed description of the normal structure of the rabbit's placenta is to be found in the papers of Duval(1, 2), Minot(6), Maximow(4, 5), and Marchand(3). The fully developed placenta of a fifteen-day embryo is a massive, cushion-like, dark red organ, occupying the mesometric side of the uterine wall. On a section through the fresh tissue, perpendicular to its free surface, the dark red superficial part, the ectoplacenta, and the pale deeper part, the maternal placenta, can be easily distinguished. The tissue fragments for explantation were taken exclusively from the ectoplacenta.

The ectoplacenta consists of a multitude of branching and anastomosing villi, whose core is formed by embryonic connective tissue with blood vessels containing circulating embryonic blood. The surface of the villi is covered by ectodermal epithelium, corresponding to the Langhans cell layer of the human placenta. Its polygonal, mitotically proliferating cells are arranged at the ends of the villi in several layers and are in turn covered by a more or less uniform layer of trophoblastic syncytium, whose basophilic protoplasm stains a deep blue with eosin-azure. The scattered oval nuclei of this layer contain but little chromatin, are pale, and never show mitoses. Between the syncytial layers irregular spaces are seen everywhere, containing circulating maternal blood.

The chief constituent parts of the maternal section of the placenta are masses of so-called glycogen cells, with a large round cell body full of glycogen and many nuclei. According to the method used, the glycogen is either seen in the form of big, specifically stained lumps or it leaves, after having

been dissolved in water, vacuoles distending the protoplasm. The blood vessels in this part of the organ are transformed into large sinusoids. Their endothelium undergoes a peculiar transformation: it forms enormous protoplasmic masses, containing huge irregular nuclei and bulging into the lumen—the so-called endovascular plasmodium. In the limit zone between the fetal and maternal parts of the placenta this hypertrophied endothelium is directly adjacent to the ectodermal trophoblastic syncytium surrounding the villi. The lumen of the blood vessels in the limit zone always contains stagnant blood with innumerable pseudoeosinophilic leukocytes. As the tissue fragments for explantation were always taken from the superficial parts of the ectoplacenta, the hypertrophied maternal endothelium could not play any rôle in the transformations in vitro as described hereafter.

THE TRANSFORMATIONS OF THE PLACENTAL TISSUE AS SEEN IN THE LIVING CULTURES

During the first hours after explantation in cultures prepared with a mixture of blood plasma and tissue extract, one can always see a considerable emigration of leukocytes, originating from the aforementioned sinusoids at the boundary between the embryonic and maternal placenta. This emigration is most intense in cultures put up with bone-marrow extract. In the presence of embryonic extract it is almost equally manifest, whereas in cultures with ovarian extract it is hardly noticeable and in pure plasma it is entirely absent or represented only by a few elements.

The emigrated leukocytes display during the first day a very active ameboid motility and are found in a wide area of fibrin surrounding the explant. Gradually, however, usually during the second day of life in vitro, they accumulate fine fat-droplets, which increase in size and fuse together to form larger drops. Coincidently, the movements are slowed down and the cells undergo degeneration. On the third or fourth day, all the emigrated leukocytes are dead.

In all cultures put up with tissue extract, a distinct liquefaction of the fibrin begins during the second day. The explant surrounds itself with a cavity; the latter acquires a round or oval shape because of the contraction of the fibrin network. The explant usually occupies in the round liquefied area an excentric position and is attached to the edge of the area. Sometimes, however, it is freely floating in the liquid. The most extensive liquefaction is always found in cultures prepared with bone-marrow extract; in cultures with ovarian extract it is insignificant; in pure plasma it is usually completely absent.

During the second day of life in vitro in some places at the periphery of the explant single spindle-shaped fibroblasts begin to invade the plasma; they originate from the mesenchyme surrounding the embryonic blood vessels in the core of the villi. They are identical with the common fibroblasts as seen in cultures of any other type of embryonic connective tissue and behave exactly in the same way. On the following day their number may slightly increase through continued emigration and partly through mitotic proliferation.

During the third day a new phenomenon may be observed —in many places on the surface of the explant massive outgrowths with rounded ends appear, showing an opaque, finely granular protoplasm with a layer of brilliant, glass-like, homogeneous ectoplasm on the outside. They are at first spherical or club-shaped. Then they grow out farther and acquire the aspect of long, broad processes (fig. 1).

In some cultures these outgrowths, after having attained considerable length, divide and branch at their ends, showing coral-like forms. In the regions of extensive liquefaction they spread on the surface of the cover-slip and may become extremely transparent. Their periphery always keeps the homogeneous and transparent condition, whereas in their central parts granular, opaque endoplasm and numerous clear nuclei are seen. In some of my cultures the length of these outgrowths attained 1 to 2 mm.

In other cases, especially in cultures without tissue extract, the proximal part of the outstretched outgrowths undergoes constriction or is pulled out into a thin thread, and finally is completely severed, so that the large protoplasmic mass with the numerous nuclei becomes independent; it shows a very distinct ameboid activity and moves away from the explant (figs. 2, 3, 4). Such isolated multinuclear cells exactly resemble huge amebae in their outer form and in the formation of pseudopodia. Their size may attain 100 to 200 μ.

Watching their movements, one can see that on the advancing edge of the huge cell body first a broad, transparent, brilliant bulging appears; when the latter reaches a sufficient size, the granular endoplasm is seen to stream in and to fill the pseudopodium completely.

The thickness of the giant cell is sometimes so considerable that it is impossible to distinguish the outlines of the nuclei in the granular endoplasm. In cases, however, where they are flattened on the surface of the cover-slip, the nuclei become very distinct and can be easily counted. From two or three in the smallest cells their number may reach a score or more in the largest ones. The granular endoplasm contains fat-droplets (fig. 6), but in a varying number.

The ameboid giant cells are very sensitive to light. If examined under the microscope with a sufficiently bright illumination, they may show, sometimes within two or three minutes, a distinct reduction of their motility; they draw in their pseudopodia and round off. If the light is turned off, the cells may recover and resume their movements. In some of my cultures showing very actively moving giant cells, the latter, after a short exposure to arc light, were found within a few minutes in a condition of complete disintegration into granular detritus. The fibroblasts of the same culture, simultaneously exposed to the same light, remained perfectly alive and continued their growth.

On the fourth and the fifth day the explant was always found to be surrounded either by solid coral-like outgrowths, as described above, or by a multitude of isolated, ameboid,

multinuclear cells. Where the latter were found in large quantities, the fibroblasts were usually absent. The development of the few fibroblasts, described for the early stages, was evidently stopped by the excessive development of the ameboid elements (fig. 5).

The large emigrated multinucleated elements, flattened on the surface of the cover-slip, undergo various transformations. Some of them divide into two equal or unequal parts with a different number of nuclei in each, and these daughter cells continue their ameboid movement. Others send out long, rigid protoplasmic processes and remain for a long time in this condition (fig. 14). Besides the fat-droplets mentioned above, in most of the cells in the granular endoplasm drops of a peculiar, pale, colorless substance develop (fig. 12); if they accumulate in large numbers, the protoplasm between them is reduced to thin partitions and the cell body acquires an alveolar structure. The cells overcrowded with the drops described, although continuing their movements for some time, finally undergo degeneration. Before the latter occurs, the large multinucleated cell body may become separated into a number of uninuclear cells, through a peculiar fragmentation occurring along the limits of the cell territories. (fig. 15).

In many cultures the explanted fragment was seen not only to give rise to massive outgrowths and large multinucleated giant cells, but also to surround itself with rapidly spreading sheets of polygonal mononuclear cells arranged epithelium-like and showing energetic mitotic proliferation (figs. 9, 11). Through secondary fusion, such epithelial cells produced giant cells, similar to the elements described above.

TRANSFORMATIONS OF THE PLACENTAL TISSUES AS SEEN IN FIXED AND STAINED PREPARATIONS

The tissue of the placenta after two days of life in vitro presents in fixed preparations profound changes when compared with a control preparation of a corresponding normal placenta. In the latter the cell layer of Langhans and the syncytium, after staining with eosin-azure, cannot be easily

distinguished from one another, both being stained blue. The tissue has a very dense and uniform structure (fig. 18). In a culture of two days, under low power, one can easily notice that the tissue consists of two parts, giving totally different staining reactions. It shows everywhere tortuous, anastomosing, broad, homogeneous strands stained pale pink with eosin; between the pink substance blue layers of cells are seen lining strands of partly degenerated mesenchyme. Examination under high power shows that the pink strands are degenerated protoplasm, containing long rows of oval, homogeneous, shrunken dead nuclei, arranged with their long axes in transverse direction and showing a pale diffuse staining and no nucleoli. In small clefts in the interior of the masses of dead protoplasm a large quantity of partly degenerated maternal erythrocytes are seen. This fact, together with the topographic relations of the homogeneous pink masses toward the cellular layers and the mesenchymal strands, proves beyond doubt that they are degenerated syncytium. The peculiar regular transverse arrangement of its nuclei and its apparently large volume in comparison with the syncytial layer in the control preparations are probably the result of swelling and coagulation of the syncytium during necrosis (fig. 19).

In later stages, after three to four days, the degenerated syncytium is becoming more and more homogeneous and stains less intensely with eosin, its nuclei also gradually losing their outlines. In cultures ten to twelve days old the completely homogeneous pale strands without a trace of nuclei can be identified as degenerated syncytium only by comparison with the early stages.

The degeneration just described, being probably a kind of coagulation necrosis, occurs rapidly, right after the explantation, and affects the whole syncytium of the explant, in the superficial and in the deeper parts of the latter as well. No signs of emigration or growth of the syncytium could be found. The changes of the syncytium just described are equally manifest in cultures put up with pure plasma and with plasma and tissue extracts as well.

THE CHANGES OF THE CELLULAR LAYER

Whereas the syncytium degenerates, the underlying cellular layer throughout the whole mass of the explant of usual size remains alive, and during the first day in vitro does not show any structural changes. Beginning with the second day, however, it begins to display a remarkable activity. The cells hypertrophy, show mitotic figures, assemble in groups, and do not keep their former arrangement in one layer, but move outward between the necrotic syncytial masses. On the free surface of the explant they invade the plasma in form of massive outgrowths as described above in the living cultures (fig. 20). In doing so, most of them are seen to produce multinucleated giant cells through fusion. If giant cells are not produced and the elements remain single, the explant surrounds itself first with the aforementioned epithelial sheets, consisting of mitotically proliferating uninucleated cells. In such membranes, as I have also mentioned in the description of the living cultures, giant cells may afterward arise through secondary fusion. In such cases the mitotic proliferation ceases (fig. 21).

The presence of tissue extracts in the medium increases the intensity and the speed of the emigration, the growth and the proliferation of the cells; it does not, however, seem to have any qualitative influence on the character of the cellular changes. The histological picture of the fixed and stained cultures put up with pure plasma did not differ from what is found in cultures prepared with tissue extracts. In the first case distinct emigration could be found only after three or four days and only single multinucleated cells were present. In cultures with bone-marrow and ovarian extracts the same condition was reached after two days, but the cells were still scarce. In cultures put up with embryonic extract, during the third day, usually numerous massive strands of multinucleated cells were present; many of these giant cells gradually became separated from the strands and scattered in the plasma.

DISCUSSION AND CONCLUSIONS

The most important phenomenon of the life of the placental tissue outside the body is the formation of giant cells of trophoblastic origin emigrating and invading the nutritive medium. As is usual for all tissue cultures in general, these elements gradually accumulate in their protoplasm fat-droplets (fig. 17); this, however, is not to be looked upon as a symptom of degeneration or even of a decrease of vitality, as the fat-containing mononuclear cells continue to proliferate mitotically, whereas the multinucleated giant cells display active ameboid movement (figs. 7, 8, 13).

Another type of inclusion, regularly found in the same cells, especially in later stages, are drops of a peculiar substance, probably of protein character; their refractive index being low, they have in the living condition the aspect of pale spherical bodies, whose size ranges from minute granules to enormous spheres, compressing the nucleus from all sides and transforming it into a small polygonal body (fig. 16). After fixation, these drops stain deeply with acid dyes; in eosin-azure preparations they are bright pink. Their relations to the fat-drops are not clear. It should be emphasized, however, that if a culture, fixed with osmic acid, be stained afterward with an acid dye—for instance, acid fuchsin—a series of gradual transitions is always found between the black fat-drops and the bright red inclusions. Sometimes a red spherical body shows at its surface a more or less distinct black coat.

The trophoblastic syncytium, being a highly specialized formation, seems to be unable to remain alive in vitro. The cellular layer, on the contrary, displays great activity. Its elements, though losing the capacity of producing anew a typical syncytium, become transformed into the multinucleated ameboid giant cells, mentioned above. The embryonic, bone-marrow, and ovarian extracts incite emigration and growth; their activity decreases in the order mentioned. However, the microscopic picture of the cultures is qualitatively independent of the various extracts.

The mesenchyme which happens to be in the deeper parts of the explant, degenerates. Its elements, occupying a peripheral position, remain alive and growing outward, give rise to cultures of common fibroblasts (fig. 10). The blood elements located in the maternal or embryonic vessels all undergo degeneration and their remnants are phagocytized by the trophoblastic giant cells.

I wish to express my gratitude to the authorities of Yale University and to Dr. R. G. Harrison, director of the Osborn Zoölogical Laboratory, for their kindness in placing at my disposal the facilities of the laboratory.

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EXPLANATION OF PLATES

Figures 1 to 15 represent photomicrographs of living cultures. Figures 16 and 17 are photomicrographs and figures 18 to 21 drawings of stained sections of fixed material.

PLATE 1

EXPLANATION OF FIGURES

Cultures of ectoplacenta of a seventeen-day (except fig. 9) embryo in pure plasma; formation and outgrowing of multinucleated ameboid cells.

1 Two days in vitro. Beginning of outgrowth. × 96.

2 Three days in vitro. A multinucleated giant cell separated from the explant and rapidly moving outward; its size at the moment of photographing was 128 × 80 μ, its thickness so considerable that no contours of the nuclei could be discerned. Some minutes after the taking of the picture, its size changed to 240 × 80 μ, and, being considerably flattened, it showed the nuclei distinctly. × 96.

3 Two days in vitro. An actively moving multinucleated giant cell with long pseudopodia; size, 320 μ. × 96.

4 Three days in vitro. Multinucleated giant cell rapidly moving away from the explant. × 96.

5 Four days in vitro. Pure culture of multinucleated giant cells, forming a broad edge around the explant; the clear spaces are occupied by nuclei. × 96.

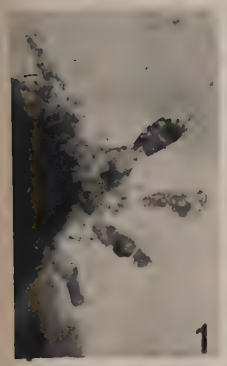
6 Same culture under high power; outlines of nuclei clearly seen. × 238.

7 Same culture; the protoplasm crowded with fat-droplets of equal size. × 238.

8 Same culture; the size of fat-droplets of equal size. × 238.

9 Culture of ectoplacenta of an eighteen-day embryo in pure plasma, three days in vitro. Epithelium-like growth, broad sheet of uninuclear cells. × 142.

10 Two days in vitro. Growth of fibroblasts. × 96.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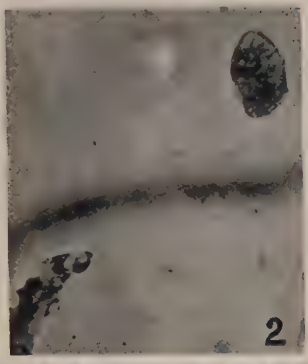
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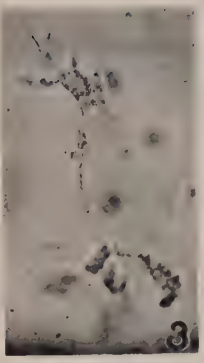
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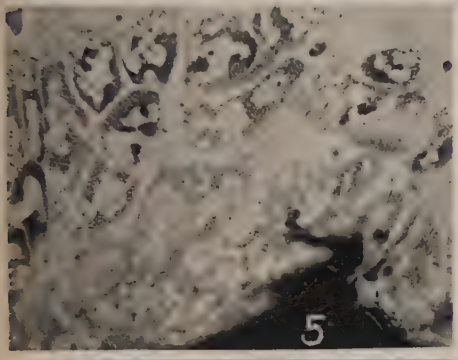
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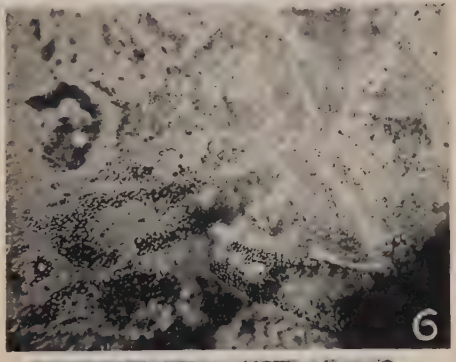
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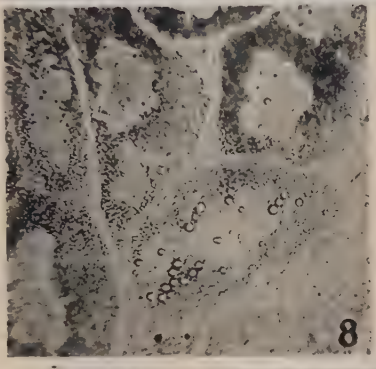
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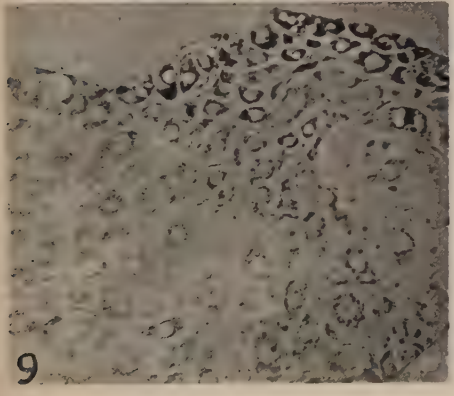
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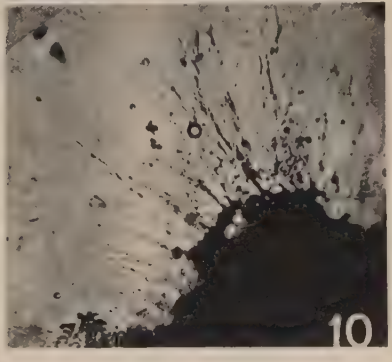
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PLATE 2

EXPLANATION OF FIGURES

Figures 11 to 15 are taken from cultures, in pure plasma, of an ectoplacenta of eighteen days.

11 Three days in vitro. The cells at the outer edge of the epithelium-like sheet contain the largest fat-droplets. × 351.

12 Five days in vitro. Large multinucleated giant cell with numerous large pale drops, staining after fixation with acid dyes. Length of the cell body, 220 μ. × 351.

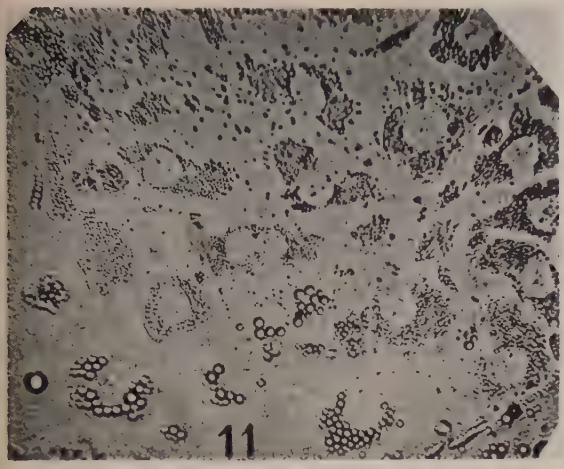
13 Three days in vitro. Large multinucleated cell with numerous fat-droplets shows very intense ameboid motility. × 351.

14 Five days in vitro. Resting giant cell with many radiating thread-like processes. × 351.

15 Five days in vitro. A multinucleated giant cell separated into uninucleated cells. × 351.

16 Five days in vitro; ectoplacenta of seventeen days, explanted into pure plasma; a giant cell similar to that on figure 12, the protoplasm containing large drops stained with eosin, Zenker-formol, eosin-hematoxylin; Zeiss achromat. obj. C, oc. 5.

17 Four days in vitro; ectoplacenta of fifteen days, explanted into a mixture of plasma and embryonic extract; fat-drops stained black with osmic acid, Zenker-formol-osmic acid (liquid of Maximow), eosin-azure; Zeiss achromat. obj. C, oc. 5.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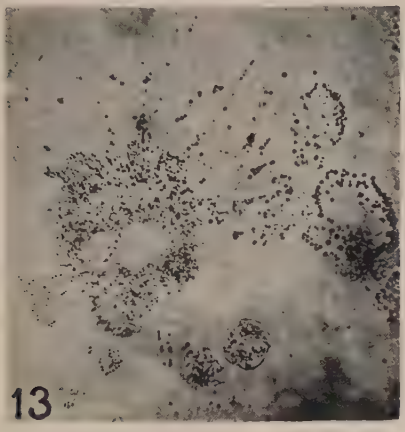
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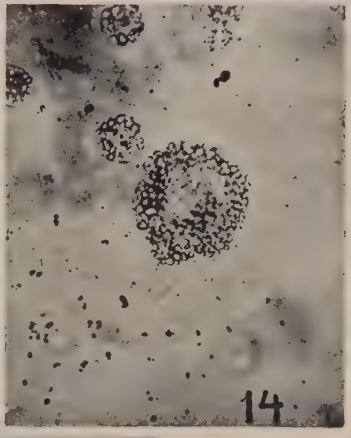
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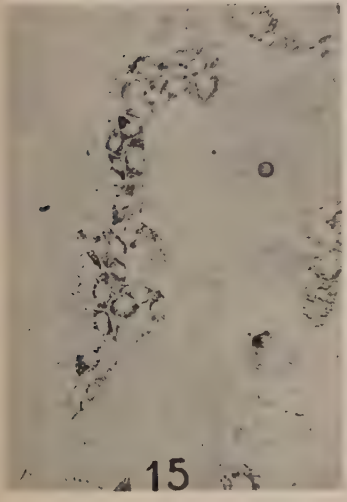
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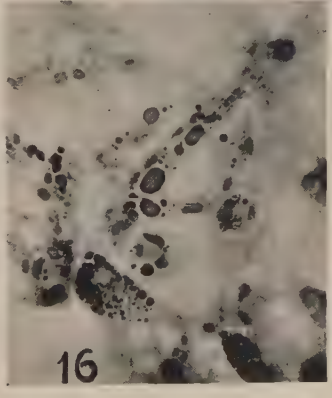
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PLATE 3

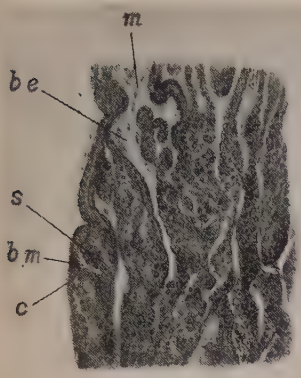
EXPLANATION OF FIGURES

18 Section of normal ectoplacenta of rabbit of fifteen days; *m*, mesenchymal core of villi; *be*, embryonic blood vessels; *c*, cellular layer of Langhans; *s*, syncytium; *bm*, maternal blood spaces; the cellular layer and the syncytium are both stained blue and are therefore difficult to distinguish under low power. Zenker-formol, eosin-azure; Zeiss achromat. obj. AA, oc. 3.

19 Two days in vitro; culture of ectoplacenta of fifteen days in pure plasma; *s*, necrotic swollen syncytium stained a pale pink with pale blue nuclei; *c*, cellular layer, stained dark blue; other abbreviations as in figure 18. Zenker-formol, eosin-azure; Zeiss achromat. obj. AA, oc. 3.

20 Six days in vitro; culture of ectoplacenta of fifteen days in plasma with embryonic extract; a strand of multinucleated giant cells, originating in the cellular layer, invades the plasma; abbreviations as in figure 19. Zenker-formol, eosin-azure; Zeiss achromat. obj. AA, oc. 5.

21 Same slide, high power; formation of multinucleated giant cells through fusion of uninuclear cells. Zenker-formol, eosin-azure; Reichert Hom. Imm. obj. 1/12, oc. 3.



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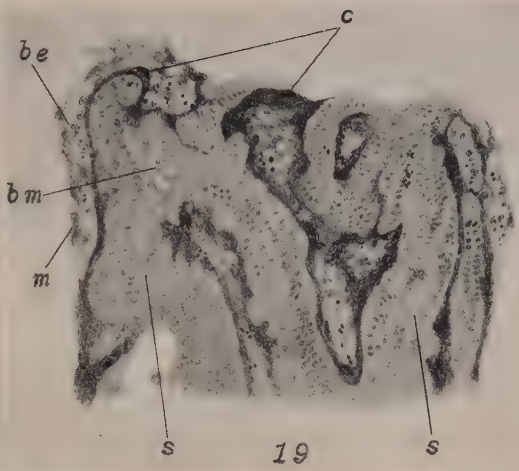

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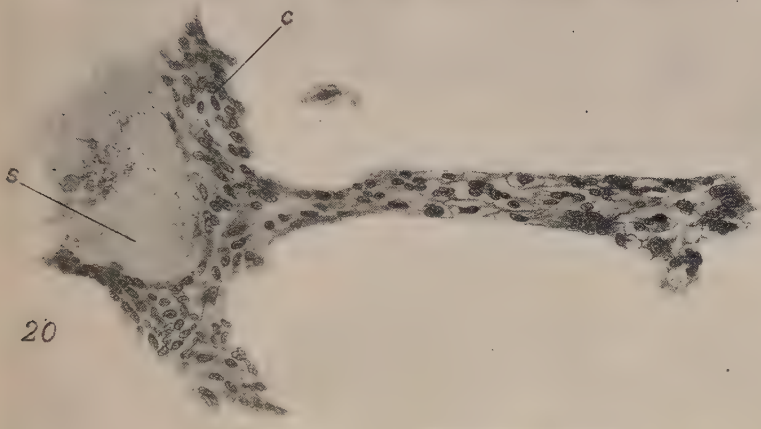

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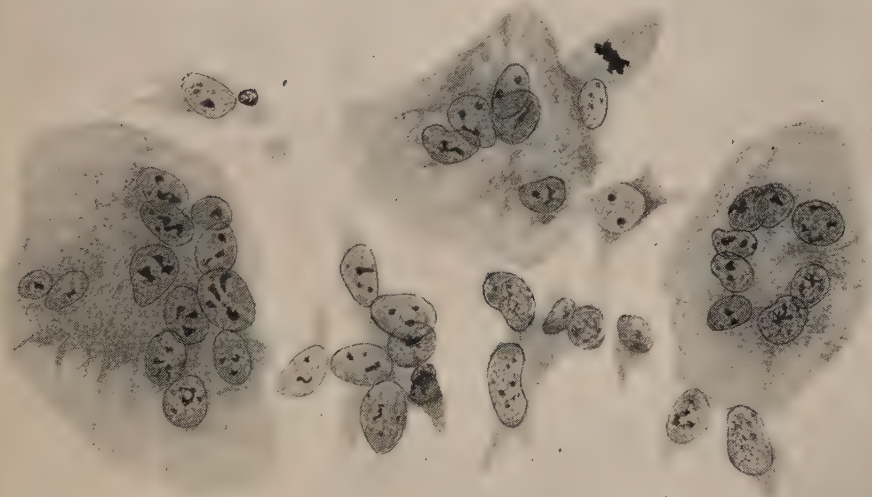

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Reprinted from the JOURNAL OF MORPHOLOGY AND PHYSIOLOGY
Vol. 40, No. 3, September, 1925

EXTERNAL REPRODUCTIVE ORGANS OF THE COMMON GRASS SPIDER, AGELENA NAEVIA WALCKENAER

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ONE TEXT FIGURE AND ONE PLATE (SEVEN FIGURES)

AUTHOR'S ABSTRACT

One of the most perfect 'lock and key' adaptations known, the external reproductive organs in spiders are subject to considerable variations. Two extreme types of female reproductive organs are described in detail, and these in turn compared with the European species of the same genus. Thus it is shown that the reproductive system in the same genus of spiders may exhibit great differences in structure while all other characters remain more or less the same, the natural conclusion being that caution should be exercised in creating new or splitting up old genera on the basis of characters of the external reproductive system. The same applies to the male.

Two extreme types of palpi are described and then compared with the palpi of the European species. In the case of the male, however, the differences seem to be of a less profound nature.

Few 'lock-and-key' adaptations in animals are as perfect as the external reproductive organs in spiders. Cross-fertilization between different species is excluded not only by the instinct of the sexes, but also, primarily and effectively, by the structure of the male and female copulatory apparatus, which is unique in the animal kingdom and distinct for each species. So permanent are the characters of these organs that they furnish the only indisputable distinction between closely allied species of all true or arachnomorph and of many mygalomorph spiders. We do not know whether spiders possessed similar structures already in the palaeozoic, since none of the few specimens in existence are sufficiently well preserved, so that even the sex cannot be recognized. But the study of coenozoic spiders, the tertiary spiders of North America and especially the amber species of the Baltic, has revealed the presence of external reproductive organs of as great complexity as in recent forms. When the value of the organs as a character was first recognized they were used

entirely for the purpose of distinguishing species. In the case of the male, a good drawing is often much more satisfactory than the most detailed description, some of the best arachnologists relying entirely on such drawings. It must be stated, however, that the type of structure is more or less characteristic of entire families and that specific differences are sometimes least apparent in the most highly specialized forms, such as the jumping spiders, for example. Several investigators have recently tried to base generic and even subfamily distinctions on the characters of external reproductive organs. This was done by Järvi in his excellent work on the vaginal system of Sparassidae and by Chamberlin for the Lycosidae and more recently for the Drassidae (Gnaphosidae). Undoubtedly, there is much in favor of such a view. The development of the external reproductive organs marks the completion of development, the attainment of maturity, even though spermatogenesis, so far as known, takes place at an earlier stage. The external reproductive organs must be therefore regarded as of more recent origin, and we should expect uniformity of structure in closely allied species and greater diversity between genera and families. Yet a word of caution in this regard seems to be not quite out of place. Careful study of variation in external reproductive organs of the same species is still lacking, while we know undoubtedly closely allied species with external reproductive organs of striking dissimilarity. Such cases occur in various families and genera, and the separation of the species into different genera on the basis of the structure of the external reproductive organs alone does not seem to be quite warranted. Thus the type of structure of the epigynum in Linyphia phrygiana and especially in Linyphia insignis is much more like that of Bathyphantes concolor than of the closer allied species of Linyphia communis, L. marginata and L. pusilla. Nevertheless, the disposition of the eyes, the shape of the sternum, the presence of spines on the metatarsi, all older characters than the external reproductive organs, are against the uniting of L. phrygiana and L. insignis under the genus Bathyphantes.

In accumulating material for a monograph on the common grass spider, Agelena naevia, I have been struck not only with the variation to which the external reproductive system is subject, but also with the great dissimilarity between the external reproductive system of A. naevia and that of the closely allied European species, A. labyrinthica. The female system of the latter has been carefully described by Engelhardt and the male palpus by Szombathy. Those of Agelena naevia have never been carefully studied, and the present investigation fills therefore a gap in our knowledge. A comparison of the two species is most instructive.

It does not require prolonged study to show that variation in size in Agelena naevia is very great. Specimens collected in the same locality, often under the same piece of loose bark, guarding their cocoons with eggs, and therefore in the same stage of maturity, are occasionally twice the size of others. The fact that two such distinguished arachnologists as Graf Keyserling and Becker have described two new species of Agelena from North America, which long since have been recognized as synonyms of A. naevia, shows that the variation in structure is also sufficient to be considered as specific in absence of intergradient forms. In his description of Agelena americana, Keyserling makes the following statement: "Agelena naevia Hentz ist dieser Art sehr ähnlich, jedoch nach der Zeichnung der männlichen Palpen die Emerton gegeben hat, entschieden als eine andere zu betrachten." Emerton was the first to notice the variation in the shape of the vulva and has given six figures showing the various types, but he has not extended his study over the complete structure of the vaginal system. He also gives six figures of the palpus showing the variation in length and structure of the embolus but does not give a description. Whether there is appreciable variation in other characters, such as spines on legs, teeth on claws, proportion of joints, etc., we do not know at all. I do not attempt to give here a statistical study of variation. Rather shall I give a description of two extreme types of female organs, compare

them with those of Agelena labyrinthica, and then describe also one type of palpus.

A few words about the technique. The vulva may be seen without further preparation, but the rest of the vaginal system is concealed from view by the abdominal wall. Engelhardt used specimens macerated in a concentrated solution of potassium hydrate at a temperature of 58° to 60°C. and stained in Congo red or eosine. I found it more convenient and simpler to study the vaginal system unstained, in a shallow dish in cedar oil, in which the object may be turned at any angle while it is being examined through a binocular dissecting microscope. The more transparent portions may be easily seen in transmitted light, while the thick-walled receptacles may be made transparent by placing the dish on a milk-white piece of glass and lighting the object from above with the aid of an electric microscope lamp and a condensing lens. Engelhardt's objection to boiling the abdomen in potassium hydrate is not substantiated by results, since no difference may be noticed between objects boiled and such prepared by prolonged soaking, as Engelhardt recommends.

The best method for studying the vaginal system in toto is therefore as follows: Boil an entire abdomen in 20 per cent aqueous solution of potassium hydrate until nothing but the chitinous structures remain. Wash in several changes of water, dehydrate, and clear gradually in cedar oil. The abdominal wall may be cut away with fine scissors as close to the edge of the vulva as practicable while the specimen is still in water. The best magnification is about 40 to 50 diameters. To make out the exact structure of the receptacles and their relation to other portions of the system, it is necessary to break the spiral fertilization duct connecting the bursa with the receptacle, after which the latter will become completely freed. The microscopic structure of the tissues and of the chitin can be studied only in sections made from fixed material, stained, and prepared in a way usual in microscopic technique. The palpus must be studied both in speci-

mens simply cleared in cedar oil and such previously boiled in potassium hydrate. The latter treatment results in considerable turgescence of the hematodocha and the consequent protrusion of the bulb with the chitinous structures which otherwise are almost hidden from view by the spiral embolus. As the swelling of the hematodocha takes place normally in life during the act of copulation, a specimen prepared by boiling in potassium hydrate corresponds closely with the natural state of the organ when in use.

The external reproductive system of the female lies in front of the genital groove into which the uterus opens. This so-called genital opening is not shown in our figures. Through it the eggs are laid. The genital opening has no other relation to the act of reproduction and is never used for purposes of copulation. Fertilization takes place before the egg is laid, while it passes through the uterus into which the right and left fertilization ducts open. There are therefore only two external openings in Agelena naevia, namely, the genital opening and the vulva.

Although of ectodermal origin, the external reproductive system of the female is situated in the body cavity and is hidden from view by the ventral abdominal wall. In a spider examined without further preparation, nothing but the vulva is visible. The abdominal wall which contains the vulva overhangs the genital groove in such a manner that it forms, below the vulva, a narrow strip bent at right angles to the surface of the abdomen. This strip contains two shallow pouches (figs. 2 and 4, *PH*) also visible without further preparation when the spider is placed head down in a deep dish with alcohol and examined in this position. The outer corners of the openings leading into the pouches are slightly produced downward, thus at two points breaking the even line of the genital groove. The openings are wide and almost circular and the pouches themselves are cone-shaped. They are not in any way connected with the rest of the external reproductive system and their function remains unknown, unless they have something to do with the process of egg laying.

Järvi, in his excellent monograph, describes similar pouches in Torania occidentalis—a spider belonging to the family Sparassidae, under the name of 'Lobaltaschen.' He does not discuss their function.

As already stated, the variations of the vulva have been correctly figured by Emerton. In some specimens it is simply transversely elliptical (fig. 2, *VUL*). In others a projection of the anterior edge partly divides the vulva into a left and a right portion (fig. 4, *VUL*). This projection or median lobe may be regarded as a homologue of a true epigynum, such as we find in other spiders. Systematists apply loosely the term epigynum to all externally visible portions of the female reproductive system in spiders—a custom rather unfortunate, in view of the fact that a distinct portion of the reproductive systems is thus confused with the entire system. The two types of vulva as represented in our figures are the extremes. Occasionally the second type may be found in small specimens, and the first in large ones, but generally the large specimens possess the second type and the small ones the first, with all possible gradations between the two types. There is, however, never a complete separation of the vulva into two independent openings.

In both types, but especially in the second, when isolated, the external reproductive system has the appearance of a very complicated organ, which, when once understood, may be reduced to a few structures. It consists of two distinct portions, one of which serves the purposes of copulation, the other those of fertilization. The former is represented by the two copulatory pouches, the atrium into which the pouches open, and the vulva. The portion which serves fertilization consists of two receptacles—two ducts connecting the copulatory pouches with the receptacles and two fertilization ducts opening separately into the uterus, close to the edge of the genital opening. The right and left halves of the apparatus are thus independent of each other except for the single

atrium, and, except for minor dislocations due to lack of space, may be considered to be perfectly symmetrical. We shall first describe the simpler type with a plain vulva as commonly met with in smaller specimens.

The atrium has the shape of an ellipsoid of variable proportions, but invariably with its long axis parallel to the genital groove. Its walls are of light yellow color. Looking into the atrium through the vulva, one sees two circular openings (fig. 2, *ABO*) leading into the perfectly transparent copulatory pouches (*BUR*). These openings are separated from each other by somewhat more than their radius, and between them form an angle of about 105°. The copulatory pouches have the shape of inverted flasks with a short and rather wide neck (*NC*). Around this neck the fertilization duct of the corresponding side is wound (*FC*). The flask itself is asymmetric, having at its bottom a diverticle, so that when viewed from this direction the bottom of the flask appears to be not circular, but bean shaped. Its walls are quite smooth. A further complication of the pouch is brought about by the presence of a blind, curved duct (*BD*) which begins at the outward corner of the diverticle, i.e., nearest the ventral wall of the abdomen, and runs in a curve toward the median plane of the body. In its last portion the blind duct lies immediately above the somewhat stouter and shorter blind duct of the receptacle for the sperm (*BC*). The function of the duct is not clear, but it is certain that it cannot be used in copulation, because its diameter is smaller than that of the embolus. The pouch alone is wide enough for this purpose. As I have always found the sperm not in the pouch, but in the receptaculum seminis (*RS*), I believe that the end of the embolus is brought into contact with the opening of the latter into the pouch (fig. 5, *OB*), and that the pouch is a true bursa copulatrix, and not a receptacle. The curved shape of the embolus and its great flexibility are in favor of such an assumption, even though direct observation cannot be made. The pouch is colorless and transparent when examined in oil, and must be rotated and examined under different angles to be fully understood.

The receptacle with its three ducts is composed of much thicker and darker chitin than either the pouches or the atrium. The canal which conducts the sperm from the pouch to the receptacle (fig. 5, *CC*) begins from the wall of the pouch not far from the neck, on the ventral surface. It runs almost in a semicircle forward and inward, ending in the bend of the receptacle. Although the wall of this duct is very thick, its lumen is considerably greater than that of the fertilization duct.

The receptacle itself is S-shaped, but so twisted that one end comes to lie in a plane forming almost right angles with the other end. The walls are very thick and covered on the inside with long, immobile spines well visible in sections. The two receptacles occupy the space between the copulatory pouches immediately in front of the atrium. Often one receptacle lies a little in front of the other, thus causing some asymmetry.

A short blind canal (*BC*), already mentioned, begins close to the connecting duct, to the inside of it. It runs in a direction, the plane of which is at right angles with the plane of the connecting duct, i.e., in a plane parallel to the plane of symmetry of the spider, and is situated immediately under the blind duct of the diverticle of the copulatory pouch.

The fertilization duct (*FC*) has an exceedingly fine, yet clearly visible lumen. It begins to the outside of the connecting duct, runs in a curve under the latter, then downward till it reaches the neck of the copulatory pouch, which it encircles, passing downward on its ventral surface, then upward on the dorsal surface, and again downward on the ventral. Thus it forms more than one coil of a spiral, appearing single on the dorsal and double on the ventral surface of the neck. It opens into the uterus (figs. 1 and 5, *OU*) not far from the genital opening, as stated above. The right and left openings are rather wide apart, being separated from each other by a space almost equal to the long axis of the vulva.

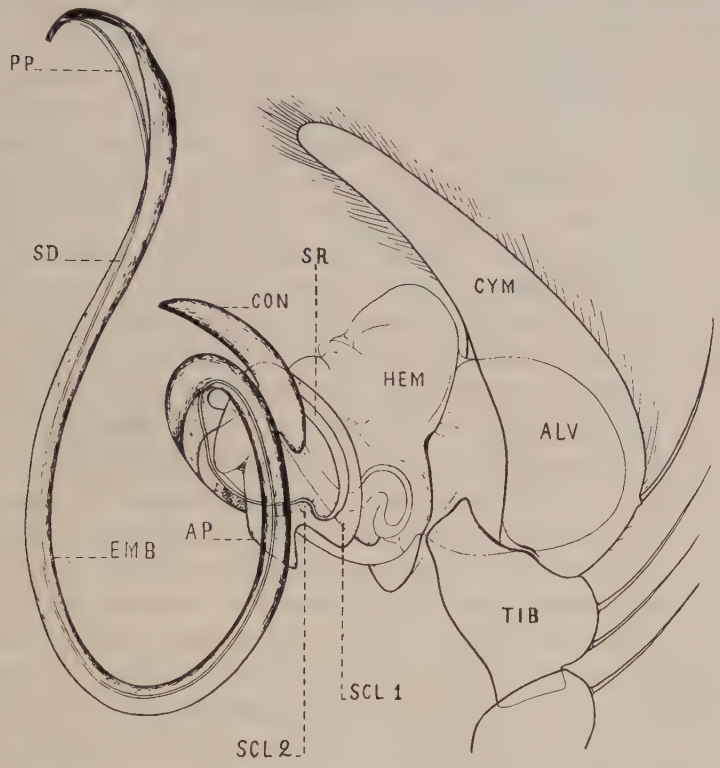
Turning now our attention to the opposite extreme of the reproductive system as observed commonly in large speci-

mens, we notice considerable modifications of all component parts (figs. 3 and 4). The neck is very long, relatively much thinner, and opens into the copulatory pouch not as in the former variety, but into the side of the pouch. The walls of the pouch and of the neck are not smooth, but pliated to such an extent that the organ appears wrinkled or shrunk, although the structure is normal and not due to the action of diffusion currents produced by the use of alcohol and oil. Nor can the pouch be stretched by an increase in internal pressure, since the wall is quite rigid. The diverticle is in direct continuation of the pouch, and is recognizable as such only because of its position and because its walls are almost free from pliations. The blind duct of the copulatory pouch is in such a position that, together with the pouch and neck, they form a sinusoid curve in which the middle portion is represented by the pouch, the outer by the neck, and the inner by the blind duct. The connecting duct of the receptacle is much heavier and longer than in the smaller variety, is bent in the shape of a wide letter S (fig. 4, *CC*), and shows a heavy thickening of the walls. The blind canal of the receptacle does not present any great modifications, but instead of lying below the blind duct of the pouch, lies above its end, often forming with the latter an X (fig. 3, *BD* and *BC*). The receptacles are still darker than in the smaller type. The fertilization canal shows often slight irregularities of curvature in its course, but also completes a turn and a half of a spiral. It may be pointed out that Engelhardt, in his description of the vaginal system of Agelena labyrinthica, applies the term receptaculum seminis III to a canal which corresponds to the blind canal of the receptacle in Agelena naevia. From a study of sections, Engelhardt describes his second and third sperm receptacles as ducts of glands, which he figures. He fails, however, to show any pores in the heavy chitin. In my own sections of Agelena naevia such pores are clearly visible, and a gland composed of numerous long cells caps the end of the blind canal into which each cell opens separately. Nevertheless, the canal as such cannot be considered as a duct of the gland,

because the gland is restricted to its tip and the walls of the canal are produced by cells which are of the common type for ectodermal structures in spiders. Engelhardt's figure 11 on plate II should be probably interpreted as representing spines, and not produced ends of glands.

In the main, Engelhardt's description of the external reproductive system in Agelena labyrinthica is correct. I had at my disposal a single specimen—the species is common in Europe, but does not occur in America—the reproductive system of which is shown in my figures 6 and 7. The first and most important difference between this species and A. naevia is in the position of the organ. Instead of being directed forward, it is directed backward, so that the copulatory pouch and the receptacle come to lie between the copulatory opening and the genital groove. Another conspicuous difference is in the absence of a common atrium and the consequent separation of the reproductive system into two independent units. In fact, of the atrium proper there is left only the roof or dorsal wall, and even this is subdivided by a narrow median longitudinal ridge. The copulatory pouch is entirely devoid of a neck and its external opening is very wide. The shape of the pouch may be best likened to that of a snail, i.e., it is helicoid with the last turn, representing the connecting canal which is in direct continuation with the pouch. The diverticle and the blind duct of the pouch are lacking. The blind canal of the receptacle or receptaculum seminis III of Engelhardt is finer and longer in proportion and is directed obliquely forward and inward instead of downward and outward as in A. naevia. The receptacle or receptaculum seminis I of Engelhardt is not twisted, but almost globular. Moreover, there is a very small blind pouch connected with it—the receptaculum seminis II of Engelhardt—situated between the blind canal and the connecting duct. This pouch is wanting in A. naevia. The fertilization canal is very short and only slightly curved. It stands in no relation whatsoever to the copulatory pouch, but runs downward and inward and opens into the uterus as in other spiders.

The external copulatory apparatus of the male Agelena naevia is also very complicated, yet once understood may be reduced to simple principles (text fig. A). The cymbium of the terminal joint of the palpus is elongated and deeply excavated in its proximal, distended portion. This excavation



Text figure A Left palpus of male Agelena naevia Walck. *ALV*, alveolus; *AP*, apophysis; *CON*, conductor; *CYM*, cymbium; *EMB*, embolus; *HEM*, hematodocha; *PP*, pars pendula; *SCL 1*, first sclerite; *SCL 2*, second sclerite; *SD*, seminal duct; *SR*, seminal receptacle; *TIB*, tibia.

or alveolus (*ALV*) hides the hematodocha when the palpus is not being used for the purposes of copulation, so that the conductor and embolus come to lie in close proximity to the edge of the alveolus. The hematodocha is clearly composed of two portions. The basal portion has the appearance of a bladder and is quite transparent. The distal portion has

the shape of a short screw with a hollow axis and a thread completing one and one-half turns. The thread is also hollow and presents different degrees of scleritization. In the specimen represented in our text figure the sclerites are limited to the ventral portion of the thread, so that there are two sclerites (*SCL 1* and *SCL 2*), while the rest of the thread remains soft and transparent. In other specimens scleritization may proceed to the dorsal surface until in some cases almost the entire thread is composed of very dark brown, hard chitin. In the trough of the thread the sperm receptacle is enclosed. Its distended blind end is bent downward in the shape of the letter S. A constant feature of the distal portion of the hematodocha is the heavily chitinized conductor (*CON*), the function of which remains uncertain. Opposite the conductor a ventral apophysis (*AP*) marks the end of the hematodocha. The embolus (*EMB*) is of very variable length, even in males of the same size. Sometimes it is too short to make a complete turn of a spiral, in other cases it is long enough to complete more than a turn and a half. Its structure has been correctly described by Comstock, who was the first to point out the presence of the so-called pars pendula or soft core of the organ, perforated by the long and extremely fine sperm duct. The embolus is therefore not a real tube, but has a slit along its entire length—the pars pendula usually concealed in the trough of the organ. The base of the embolus is considerably widened and clearly set off from the transparent hematodocha. The surface of the embolus is fluted. At the end the embolus becomes suddenly thin and curved in a plane which is usually at right angles with the main spiral. The embolus, however, is very flexible, and when the hematodocha is turgescent the spiral is wider than in the quiescent state. The opening of the duct is on the tip of the embolus, the tip being not rounded, but squarely truncated. The duct may be followed without difficulty through the entire length of the embolus. The proximal end of the duct lies in the trough of the second sclerite of the hematodocha, where it suddenly dips downward before it becomes enlarged and assumes the aspect of a receptacle.

As in the case of the female, all parts of the male external reproductive organs are subject to considerable variation. Only the general plan remains the same in individuals of all sizes, so that it is impossible to say whether there is a strict relationship between the length of the embolus and the length of the neck of the copulatory pouch. Even the shortest embolus seems to be still considerably longer than the neck, thus assuring the possibility of successful copulation.

In its main features the palpus of Agelena labyrinthica resembles that of our A. naevia. At the same time the differences are also striking. Thus the embolus is quite small when compared not only with the embolus of Agelena naevia, but with the hematodocha and its scleritized portions in same palpus of A. labyrinthica. Although completing more than one turn of a spiral, the embolus is distinctly smaller than the thread of the distal portion of the hematodocha, the diameter of the latter being at least twice that of the former. The distal portion of the hematodocha has the shape of a screw, but its axis, instead of being cylindrical, is barrel-shaped. The conductor is attached to the hematodocha, not with its base, but near its center, so that it resembles a pickax, with one end pointed and the other bluntly rounded. There is, besides, a second conductor which is possibly homologous with the ventral apophysis of A. naevia, but which is strongly developed and so situated that, together with the dorsal conductor, they form a letter X. A superficial comparison of the palpi of the two species undoubtedly would emphasize the diversity of structure, but the modification is, nevertheless, much greater in the female than in the male and involves much more important changes.

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PLATE 1

EXPLANATION OF FIGURES

1 Dorsal view of the external reproductive system of a female of Agelena naevia Walck. Small variety.

2 Ventral view of the same.

3 Dorsal view of the external reproductive system of a female of Agelena naevia Walck. Large variety.

4 Ventral view of the same.

5 Diagram of the receptacle and its ducts in Agelena naevia Walck. Small variety.

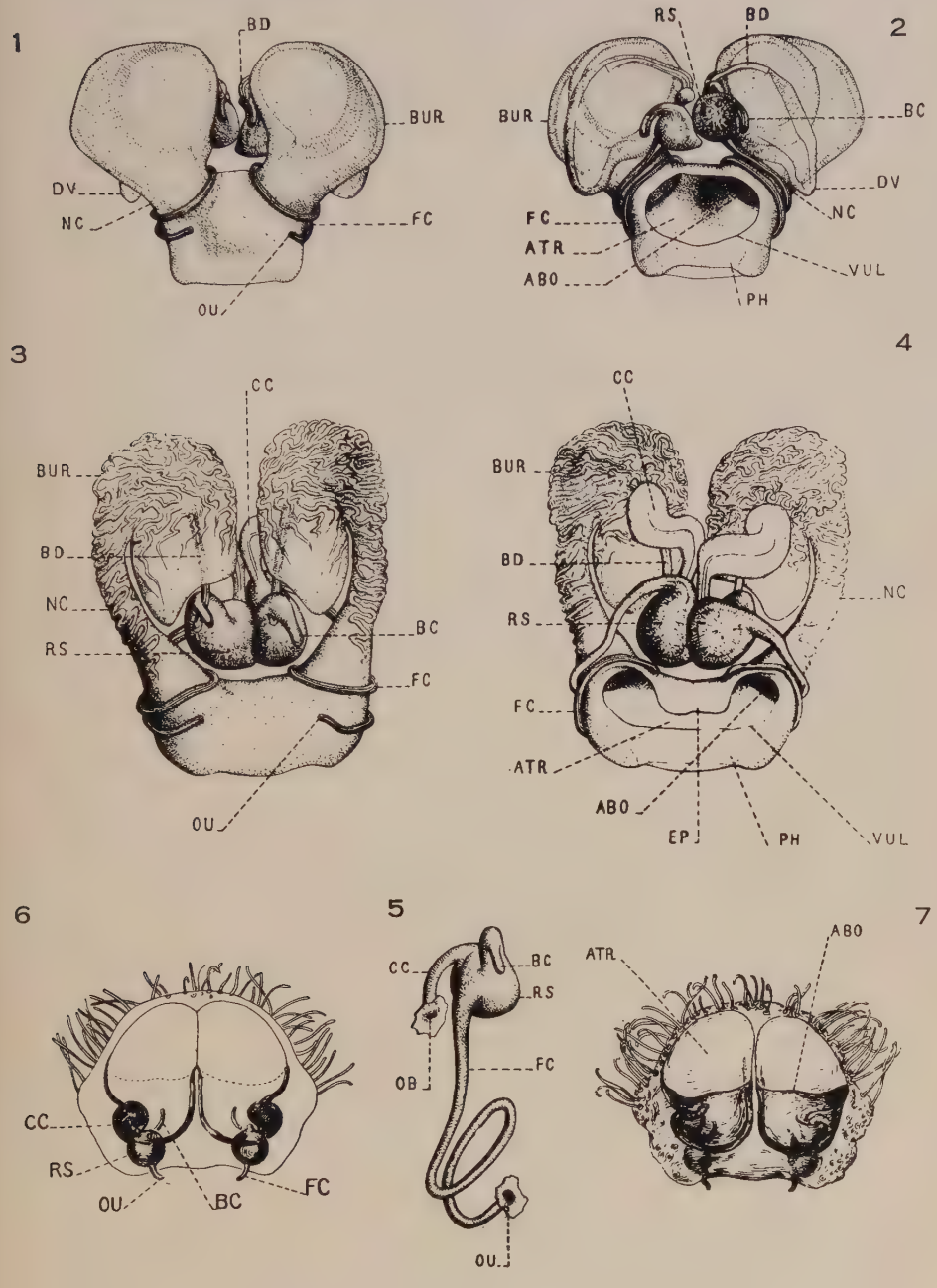
6 Dorsal view of the external reproductive system of a female Agelena labyrinthica Clerck.

7 Ventral view of the same.

ABBREVIATIONS

ABO, atriobursal orifice
ATR, atrium
BC, blind canal of the receptacle
BD, blind duct of the diverticle of the bursa
BUR, bursa copulatrix
CC, connecting canal leading from the bursa to the receptacle
DV, diverticle of the bursa
EP, epigynum
FC, fertilization canal
NC, neck of bursa
OB, opening of connecting canal into bursa
OU, opening of fertilization canal into uterus
PH, pouch
RS, receptaculum seminis
VUL, vulva

ALEXANDER PETRUNKEVITCH



Reprinted from Journal of the New York Entomological Society,
Vol. XXXIII, September, 1925.

NEW ERIGONINAE FROM TENNESSEE

By Alexander Petrunkevitch
Professor of Zoology in Yale University

The species described in this paper were collected by Mr. S. E. Crumb in Clarksville, Tennessee, in 1921. They form part of the collection made on 50 plats of one square yard each and sent to me for identification by Dr. L. O. Howard, of the Bureau of Entomology. By agreement with Dr. Howard the complete list of arachnids of this collection and the report of their distribution according to plats has been placed in his hands, and there is no further need to give the list here. Since six of the species, all belonging to the sub-family Erigoninæ, proved to be new, I thought it advisable to give careful descriptions which would not only permit identification, but help in the understanding of the classification of this group.

The classification of the Erigoninæ is still in an unsatisfactory state. Species are often easier identified than genera, especially in the case of males, when accompanied by good drawings. No comprehensive work on the group exists and the opinion of arachnologists as to the value of different characters is still divided. Simon was the first to give detailed descriptions of French Erigoninæ in his excellent work, "Les Arachnides de France." He made use chiefly of characters derived from the structure of the sternum, the fangs, the eye group and the spines on legs. Later in his great "Histoire Naturelle des Araignées" he reclassified the Erigoninæ, making use of a number of characters, but still basing his classification on structures mentioned. Dahl was the first to pay special attention to the distribution of auditory hairs or trichobothria, a character the value of which was later recognized by Chyzer and Kulczynski. Nevertheless the use of this character has not found general recognition, partly perhaps because Simon did not consider it of great value and thought that trichobothria are easily overlooked and as easily broken off and lost. The latter statement does not seem to find

support in fact, for it is easy to show that trichobothria are much less subject to injury and loss than are spines and bristles. Moreover, they can be detected without any difficulty under a binocular with sufficient power and with good artificial illumination. On the whole they seem to me to be not only quite reliable characters, but in many ways more reliable than the composition of the eyegroup which, as is well known, is greatly affected by the eyetubercle in some males, so that males do not quite agree with females of the same species in this respect. I have lately examined a number of genera with the result that I cannot recommend too strongly the use of trichobothria as distinctive characters.

Diplocephalus crumbi new species. Male.

Total length 1.58 mm., Carapace 0.75 mm. long, 0.65 mm. wide. Thoracic groove wanting. Clypeus about as high as the quadrangle. Head elevated in the shape of a cube carrying the posterior middle eyes. Foveæ wanting. Margins of carapace smooth, plain. First row of eyes strongly recurved, second row slightly recurved. Viewed from in front first row straight, second row curved downward. Side eyes contiguous, ALE slightly larger than PLE. Eyes of first row equidistant, separated by less than the diameter of the AME. Posterior middle eyes closer together than AME, slightly larger than PLE and only very slightly smaller than AME. Quadrangle wider in front than behind, as long as wide behind. Cheliceræ long and powerful, lower margin with three equidistant teeth, upper margin with three teeth of which the distal one is near the base of the fang and separated from the median tooth by double the space between the latter and the proximal tooth. Anterior surface of cheliceræ with a tooth situated a little in advance of the median tooth of the upper margin. Fangs normal. Sternum triangular, broadly truncated in front, rounded behind between the hind coxæ which are separated from each other by more than their width, slightly wider than long in the ratio of 12/11. Lip occupies middle third of the anterior truncature of the sternum, strongly rebordered, its anterior portion triangular, extending beyond middle of maxillæ which are long, with subparallel sides, converging. Legs slender, without spines. Anterior tarsi slightly longer than half of metatarsi (13/22), metatarsi slightly shorter than tibiæ (22/27). Posterior tarsi 9/16ths of metatarsi, posterior metatarsi 16/19ths of tibiæ. Upper claws of first leg with several strong teeth, those of fourth leg smooth. Third claw smooth. Trichobothria on first leg: 1 on femur near proximal end, 2–2–1 on tibia (first pair close to proximal end, the single one a little before middle), 1 on metatarsus 1/3 from proximal end. A "drum" in middle of tarsus. (The drum is a sense-organ of unknown function.) Trichobothria on fourth leg: 1–2–2–1 on tibia, the last one in middle, none on metatarsus or any of the other joints.

Palpus as figured (Figures 1 and 2). Tibial apophysis long, thin and slightly curved outward, with rounded end. Bulb very large, almost globular. Embolus in the shape of a question mark. Dorsal median line of patella of palp twice as long as the greatest width, strongly convex. Ventral median line slightly longer than greatest width, straight. Cymbium of a very peculiar shape. Its basal half bearing the hæmatodocha is ovoid, then follows a narrow stem which ends in a curved, spoon-shaped dilation. Few short hairs on patella, several long, curved hairs on tibia.

Abdomen eggshaped, overlapping carapace, slightly coriacous. Color in alcohol: Carapace rufous, median eyes surrounded by black rings. Legs yellowish brown, without markings, darker than abdomen. Cheliceræ, lip, maxillæ and sternum of the same color as carapace, sternum with darker margins. Bulb of palpi darker than legs, grayish-brown. Abdomen yellow above, with 4 pairs of transverse narrow white lines in posterior third, below grayish-yellow. Spinnerets with smoky tips. Hair on sternum long, on abdomen short and sparse, dark in color. Three males.

Erigone clarksvillense new species. Female.

Total length 2.7 mm. Carapace strongly emarginate behind. Measured in median line only 1.080 mm. long, but fully 1.224 mm. long when measured to greatest extension of posterior edge. Width between 2d and 3d pair of legs 0.936 mm. Width in front 0.396 mm. Abdomen 1.944 mm. long, soft, considerably overhanging carapace, reaching quite to the cephalic portion, sparsely covered with hair. Edge of carapace normal, neither rebordered, nor toothed. Head low. First row of eyes recurved. AME smaller than ALE, separated from each other by their radius, more than by their radius from ALE (by the radius of the latter). Second row viewed from above straight, slightly longer than anterior row. Posterior eyes equidistant, separated from each other by almost their diameter. Lateral eyes contiguous. Quadrangle narrower in front than behind, as long as wide behind. Viewed from in front first row straight, by lower edges, second row strongly curved downward. Clypeus 4/6ths the length of the quadrangle, plane, vertical. Sternum triangular, as wide as long, very slightly narrowed in front, with straight anterior margin. Posterior end of sternum extending into a widened portion which is continuous with the carapace, so that the exact length and shape of the sternum cannot be ascertained. Viewed in a certain position, however, when the posterior extension referred to is invisible, the sternum appears truncated straight between the hind coxæ. Lip strongly rebordered, narrower at base, occupying middle third of anterior truncature of sternum. Maxillæ large, longer than wide, slightly converging. Cheliceræ powerful, their outer outline parallel, margins strongly oblique. On front surface near outer edge a row of five very small granules with a tiny spine on each, absent in one of the specimens. Similar granules near upper margin and a small bristle near middle of inner edge. Upper margin with 5 strong teeth, lower margin with 4 smaller

teeth. Thoracic groove longitudinal. On head in front of groove three median hairs, equidistant, curved forward. Hind coxæ separated by slightly less than their width. A median dorsal spine at end of each patella and on all tibiæ 1/3d from base. A ventral spine near end of femur. Legs strong. Tarsi of first leg 15/24ths of metatarsi, tarsi of fourth leg 13/24ths of metatarsi. Metatarsi only by 1/24th shorter than tibiæ. Upper claws on all legs with a row of small teeth increasing in length distally. Third claw with a single strong tooth. Palpus without claw. Trichobothria on first leg: 1–1–1–1 on tibia and same on fourth leg, but there is also a small "drum" in middle of fourth tarsus.

Color in alcohol: Carapace yellow-brown with indistinct black marginal line. Legs of the same color as carapace. Cheliceræ slightly darker. Sternum much darker, greyish-brown. Abdomen grey with a darker median line and four darker indistinct transverse lines in posterior half. Sides of abdomen with numerous indistinct yellowish spots. Epigynum dark, prominent. (Figures 3 and 4.) Three females.

Tennesseellum new genus.

Abdomen soft. Tracheal spiracle large and placed considerably in advance of the spinnerets. Carapace flat. Anterior row of eyes recurved, posterior row slightly procurved. Quadrangle as long as wide. Clypeus narrow, inclined. Sternum convex, broadly truncated between hind coxæ, slightly longer than wide, reticulated. Upper margin of cheliceræ with 1 tooth, lower margin with two teeth. Legs short and slender. Tarsi longer than half of metatarsus, the latter almost as long as tibia. First metatarsus with a trichobothrium 1/3 from base, fourth metatarsus without trichobothria. Typus *T. minutum.*

Tennesseellum minutum new species. Female.

Total length 2.232 mm. Carapace 0.756 mm. long, 0.540 mm. wide, rounded in front, 0.252 mm. between outside edges of ALE which occupy the entire width. Longitudinal groove well marked. Carapace flat, head not elevated, with a row of 3 median bristles, first immediately in front of longitudinal groove, third near the eyes. Abdomen soft, 1.440 mm. long, barely overlapping carapace, so that in certain positions the entire petiolus is visible. The tracheal spiracle appears as a distinct transverse line considerably in advance of the spinnerets, the distance between the genital groove and the spiracle being double that between the spiracle and the anterior spinnerets. Anterior row of eyes recurved. AME slightly smaller than ALE. Posterior row slightly procurved. Eyes equidistant, separated by less than their diameter. Quadrangle very slightly wider behind than in front, as long as wide behind. AME overhanging the clypeus which is slightly inclined forward and only ½ the length of the quadrangle. Viewed from in front anterior row is very slightly curved downward. AME separated from each other by their radius, slightly more than by their radius from the ALE which are a

little larger. Lateral eyes contiguous. Chelicerae weak, margins but slightly oblique, upper margin with a single tooth, lower margin with two teeth. On outer surface of chelicerae a well developed stridulating ridge. Lip wider than long, strongly rebordered. Maxillae short, stout. Sternum convex, broadly truncated between hind coxae, reticulated, slightly longer than wide (in the proportion of 13/11). Legs short and slender. Tarsi of first leg 50/73 ds of metatarsi, and these 73/75 ths of tibiae. Tarsi of fourth leg 46/80 ths of metatarsi and these 80/87 ths of tibiae. A slender spine below near distal end of each femur, and above on first tibia. A still weaker spine above near base of first tibia, while on other legs it is not different from other hairs. Upper claws on all legs with a few very small teeth, third claw with a single small tooth. Palpus without claw. Trichobothria on first leg: 1–1 on tibia and 1 on metatarsus 1/3 d from base. On fourth leg only 1–1–1 trichobothria on tibia, none on metatarsus. No "drum" on tarsi. Color in alcohol: Carapace, sternum and legs dark yellow, but first three pairs of femora and the distal end of the palpi are dark brown. Abdomen dark grey with indistinct transverse striation on sides. A single female. Figure 5.

Oedothorax howardi new species. Female.

Total length 2.448. Carapace 1.044 mm. long, 0.792 mm. wide between 2d and 3d pair of legs, 0.360 mm. wide in front, rounded behind with median semicircular emargination. Longitudinal groove barely discernible. Surface of carapace evenly reticulated. Lateral eyes on a tubercle. Clypeus vertical, plane, reticulated, not quite as high as quadrangle. First row of eyes straight by anterior edges, second row strongly procurved. Both rows equally long (Figure 9). Lateral eyes contiguous. ALE much the largest, AME the smallest. Posterior eyes equal in size, equidistant, separated from each other by almost their diameter. AME separated from each other by 1½ (one and a half) their radius, and by almost their diameter from ALE which are almost twice as large. Chelicerae strong, short, with oblique margins. Lower margin with five equidistant small teeth, upper margin with five strong teeth of which the second proximal tooth is the longest. Fang with distinctly serrated outer edge (Stridulating organ?). Lip wider than long, occupies middle third of anterior margin of sternum. Maxillae widened in front. Sternum roughly reticulated, slightly convex, triangular, almost as wide in front as between 2d and 3d coxae. Anterior margin of sternum slightly procurved. Posterior end procurved between hind coxae, rounded. Hind coxae separated by their width. Legs short, stout. All tarsi only slightly shorter than metatarsi. A median dorsal spine at end of patella and a little before middle of tibia on all legs. One ventral spine near distal end of anterior femora. Upper claws on all legs with a few fine teeth, third claw with 2 very fine teeth. Palpus without claw. Trichobothria on first leg (Figure 7): on tibia 1–1–1–1, none on other segments. Trichobothria of fourth leg (Figure 8): on tibia 1–1–1–1, on metatarsus 1, very long, in middle. No "drum" on tarsi. No hairs on carapace. Abdomen soft, ovoid,

overlapping carapace, sparsely covered with minute hairs. Color in alcohol. Carapace, sternum, lip and maxillæ dark brown, legs yellow, almost orange. Abdomen dark grey-brown. Epigynum as figured (Figure 6). Three females.

Caseola howardi new species. Female.

Total length 1.872 mm. Carapace 0.756 mm. long, 0.576 mm wide, little narrowed in front, so that the front is much wider than the eyegroup. Two small median bristles on head, first just at the posterior limit of the head, second slightly in front of first. Carapace smooth, without hair. First row of eyes straight, second strongly procurved, slightly longer. Lateral eyes contiguous. Viewed from in front first row is distinctly bent downwards, AME much smaller than ALE, separated by their diameter both from each other and from the laterals. Posterior eyes equal in size to ALE and equidistant. Quadrangle narrower in front than behind, as long as wide behind. Clypeus as high as quadrangle, simple, slightly oblique, with anterior eyes overhanging it. Lip much wider than long, rebordered. Maxillæ inclined over lip, curved, inner angle almost 90 degrees. Cheliceræ parallel by outer edges. Margins oblique. Upper margin with 5 strong teeth, lower margin with 5 weaker teeth. Sternum triangular, produced between hind coxæ where it is squarely truncated, convex, punctate, as wide as long, not narrowed in front. Legs short, first pair considerably shorter than fourth. A dorsal spine at end of all patellæ, and a ventral bristle near end of all femora, assuming the appearance of a slender spine only on femora of fourth pair. No spines elsewhere. Metatarsi much shorter than tibiæ. First tarsus 42/50 ths of metatarsus, fourth tarsus 40/51 sts of metatarsus. Upper claws on all legs with a series of strong teeth, third claw with one minute tooth. Palpus without claw. Trichobothria of first leg: on tibia 1–1, on metatarsus 1 in middle. Trichobothria of fourth leg: on tibia 1–1–2–1–1, none elsewhere. No "drum" on tarsi. Abdomen soft, 1.189 mm. long, slightly overhanging carapace. Color in alcohol. Carapace, legs, palpi and maxillæ yellow. Sternum yellow with smoky edge, minutely mottled with black. Abdomen uniformly greyish above and below. Epigynum as figured (Figure 10), almost colorless, except for chitinized median lines. One female.

Gongylidiellum tennesseense new species. Female.

Total length 1.584 mm. Carapace 0.648 mm. long, 0.504 mm. wide, little narrowed in front which is wider than eye area. Longitudinal groove barely noticeable. Head not elevated. Clypeus ¾ as high as quadrangle. First row of eyes slightly recurved, second row slightly procurved. Laterals contiguous. AME smallest, ALE slightly larger than PLE. AME in a transversely oval black spot, sub-contiguous, slightly but distinctly separated from ALE. PME separated by their diameter from each other and by less than diameter from PLE. Quadrangle narrower in front than behind, as long as wide behind. Cheliceræ weak. Upper margin with 5 teeth, lower

with 4 small teeth. Sternum flat, triangular, longer than wide, squarely truncated between hind coxæ. Legs slender, fourth leg the longest. First tarsus 41/52 ds of metatarsus, first metatarsus 52/62 ds of tibia. Fourth tarsus 37/54 ts of metatarsus, fourth metatarsus 54/68 ths of tibia. Spines very slender. One spine below, near distal end of all femora, while elsewhere legs are covered only with hair. Upper claws on all legs with a few small teeth, third claw with a single small tooth. Palpus without claw. Trichobothria of first leg: on tibia 1–1–1, none elsewhere. Trichobothria of fourth leg: on tibia 1–1–1, none elsewhere. No "drum" on tarsi. Abdomen soft, ovoid, 0.972 mm. long, overhanging carapace a little. Color in alcohol. Carapace, cheliceræ, maxillæ and legs yellow. Sternum much darker, mottled with black. Abdomen greyish yellow. Epigynum as figured (Figure 11). One female.

EXPLANATION OF PLATE VIII

Fig. 1. *Diplocephalus crumbi* n. sp. Left palpal bulb from above, showing the tibia with long bristles and the long, curved tibial apophysis.

Fig. 2. Same palpal bulb from below, showing curved embolus, cymbium widened at the end, and the tip of the tibial apophysis behind the hæmatodocha, which is of an unusually large size and almost spherical in shape.

Fig. 3. *Erigone clarcksvillense* n. sp. Epigynum in profile.

Fig. 4. Same epigynum in surface view.

Fig. 5. *Tennesseellum minutum* n. g., n. sp. Epigynum.

Fig. 6. *Oedothorax howardi* n. sp. Epigynum with the trapeze-shaped grey area surrounding it.

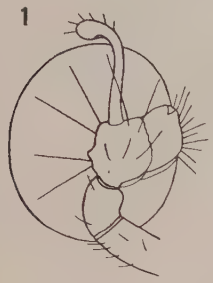
Fig. 7. *Oedothorax howardi.* First leg, showing the proportions of the segments, spines and trichobothria.

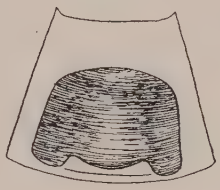
Fig. 8. *Oedothorax howardi.* Fourth leg, showing same structures and the trichobothrium of the metatarsus.

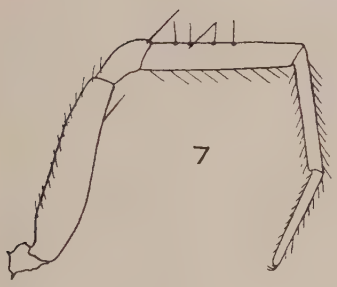
Fig. 9. *Oedothorax howardi.* Eyegroup and clypeus. In the position shown only anterior middle eyes appear as circles, although all eyes are perfectly round when viewed in the proper direction.

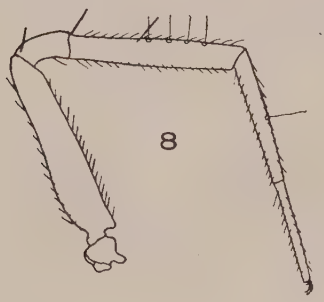
Fig. 10. *Caseola howardi* n. sp. Epigynum.

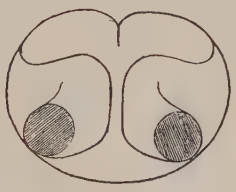
Fig. 11. *Gongylidiellum tennesseense* n. sp. Epigynum and genital groove.











ERIGONINAE

Reprinted from The American Journal of Anatomy
Vol. 36, No. 1, September, 1925

AUTOPLASTIC AND HOMOPLASTIC PARATHYROID TRANSPLANTATION[1]

W. W. SWINGLE AND J. S. NICHOLAS

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FOUR TEXT FIGURES AND ONE PLATE

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INTRODUCTION

During the course of an extensive morphological and physiological investigation of the parathyroids of the cat, several autoplastic transplants of the glands were made, and it was noted that none of the grafted animals developed fatal tetany following removal of the thyroparathyroid apparatus. We were not at that time concerned with transplantation, and little attention was given to this phase of the work. During the past year, however, our time and attention have been devoted almost exclusively to grafting experiments, and this paper deals with the results of thirty-four autoplastic and

[1] The experimental work was done at the Osborn Zoölogical Laboratory, Yale University.

homoplastic parathyroid transplantations in the cat. We were particularly interested in homoplastic grafting, to satisfy ourselves whether or not this type of transplantation could be successful in mammals.

The work of Harrison ('07, '18, and '21) and his students has clearly shown that homoplastic grafting of tissues and organs is feasible in amphibians, at any rate if performed during the course of embryonic development. More recently, Harrison ('24) has demonstrated that heteroplastic limb transplantation can be successfully performed in this vertebrate group, and Burns ('24) has reported successful heteroplastic parabiotic grafts of salamander larvae.

Aside from the work on Amphibians, where there can be no question that homoplastic and even heteroplastic grafting is attended with a high degree of success, considerable evidence has accumulated indicating that even in mammals the homoplastic method can be successfully employed with certain tissues. Authentic reports of successful skin and bone grafts have been reported for years, and the recent work of Steinach ('13), Sand ('19), and Moore ('19, '20), on testicle and ovarian transplantation, indicate that it is possible to obtain permanent homoplastic grafts of these structures in mammals.

A second consideration which led us to undertake the present work was the fact that during the past twenty years numerous papers have appeared, detailing successful experiments on homoplastic parathyroid transplantation in man; that is to say, successful in so far as the symptoms of tetania parathyropriva were alleviated or permanently cured; the fate of the graft in practically all of the cases remaining unknown.

These papers on human parathyroid grafting are of considerable interest, both from a theoretical and a clinical standpoint. Our work on the cat ('23, '24) showed that at least 35 per cent of these animals possess accessory parathyroids, and we suspect that if other mammals, including man, were carefully examined for accessories, that numerous individuals

would reveal them. Consequently, we were curious as to the results of autoplastic and homoplastic grafting in such a species as the cat, where such a large number of animals have accessories. On theoretical grounds, it was considered likely that the per cent of cats surviving thyro-parathyroidectomy would be abnormally high following gland transplantation, because the grafts would probably tide the organism over the first few critical days following operation, thus allowing time for any accessories that might be present to function adequately for the needs of the organism. If such proved to be the case, the experiment might shed some light upon the reason for the remarkable successes reported, following homoplastic and even heteroplastic parathyroid transplantation in man.

LITERATURE

The literature upon parathyroid transplantation is extensive, but to the best of our knowledge very few investigators have employed cats as experimental material. The dog, rat, and rabbit have been utilized by most workers. It would be of little profit here to review in extenso the literature on parathyroid grafting, so we shall confine our attention to those papers dealing with the cat.

Morel ('12), in his excellent monograph, "Les Parathyroides," has fully covered the literature on parathyroid grafting up to 1911, and lists in tabular form the principal results of each investigation.

So far as we are aware, but four investigators besides ourselves have transplanted the parathyroids of cats. Von Eiselberg ('92) autoplastically transplanted fresh thyroid glands of cats into the abdominal fascia following thyroidectomy, and observed that tetany was thus prevented; subsequent removal of the transplanted tissue resulted in fatal tetany. At the time this experiment was performed von Eiselberg had no idea of the relation of the parathyroids to tetany, and considered that he was dealing solely with the thyroid. We now know that the grafted thyroid must have contained parathyroid tissue.

TABLE 1

Summary of the literature on homoplastic parathyroid transplantation in man

CASE	REFERENCES	CONDITIONS FOR WHICH GRAFT WAS USED	LOCATION OF GRAFT	CONSTITUTION OF THE TRANSPLANT	RESULT
1	Garre-Krabbel, Beitr. z. kl. Chir., Bd. 52, H. 2, 1911	Chronic postoperative tetany	1. Intratibial	Four small pieces of a goiter removed for Basedow's disease	Very doubtful
			2. Preperitoneal	One parathyroid removed from a goiter as above	Improvement very marked
2	Ibid.	Idiopathic tetany	Preperitoneal	One parathyroid removed from goiter case	Temporary improvement
3	v. Eiselberg, Zeit. f. Chir., 1908	Postoperative tetany	Preperitoneal	One parathyroid from goiter case	Improvement
4	Kocher, Korr. Bl. f. Schweizer Aerzte, 1909	Postoperative tetany	Intratibial	Portion of a goiter	Improvement
5	Czerny, Zent. Bl. f. Chir., 1908	Postoperative tetany	Intrasplenic	Probably a thyro-parathyroid graft	Improvement
6	Böse, Wien. med. Wochen., 1909	Postoperative tetany	Intra-abdominal	One parathyroid from a goiter case	Complete and permanent recovery
7	Pool, Ann. of Surg., 1907	Postoperative tetany	1. Subcutaneous in abdominal region	Five parathyroids secured from accidental deaths in three subjects	Progressive improvement with complete recovery
			2. Auricular		
8	Danielson, Beitr. z. kl. Chir., 1910	Postoperative tetany	Preperitoneal	Two parathyroids recovered from goiter case	Gradual recovery which was permanent
9	v. Eiselberg, Verh. d. Deutch. Ges. f. Chir., Bd. 37, 1908	Postoperative tetany (two cases)	Preperitoneal	Parathyroids recovered from goiter cases	Recovery
10					
11	Pool and Turner, Ann. of Surg., vol. 56, 1912	Postoperative tetany	Preperitoneal	Parathyroids recovered from goiter cases	Recovery
12	Stenvers, Nederl. Tydschr. v. Geneeskunde, 1917	Postoperative tetany	Preperitoneal	Three parathyroids recovered from goiter case	Recovery
13	Borchers, Zent. f. Chir. Bd. 46, H. 1, Nr. 3, 1919	Postoperative tetany	Preperitoneal ext. oblique	Two parathyroids implanted	Permanent improvement

14 15	Ibid., Nr. 46, 2te H.	Deficiency during pregnancy (two cases)	Preperitoneal	Parathyroid tissue from infant	1. Latent tetany with improvement 2. Partial alleviation with recovery
16	Landois, Zent. f. Chir., Bd. 47, H. 1, Nr. 4, 1920	Postoperative tetany	Rectus sheath	Three parathyroids from goiter cases	Temporary alleviation. Death from tetany
17 18	Flörcken and Fritzsche, Zent. f. Chir., 1920	Postoperative tetany	Int. oblique	Three parathyroids	Latent
19 20	Thierry, Münch. med. Woch., Nr. 20, 1919	Postoperative tetany (two cases)	Preperitoneal	Parathyroid tissue recovered from goiter cases	Partial recovery Died of tetany
21 22	Jaeger, Zent. f. Chir., Nr. 23, 1920	Postoperative tetany (two cases)	Preperitoneal	1. Three parathyroids from newborn 2. Four parathyroids from newborn	Temporary recovery only
23	Brown, Ann. of Surg., vol. 75, 1922	Postoperative tetany	Preperitoneal (three successive graftings	1. Three parathyroids from goiter cases 2. Fresh beef parathyroid 3. Four parathyroids from an accidental death	No effect No effect No effect—patient died

According to Morel ('12), Walbaum ('03) made autoplastic grafts of two parathyroids of the cat, placing the glands 'sous la séreuse stomacale.' He later destroyed the two remaining parathyroids, and his animals died of tetany or cachexia. Evidently the autoplastically grafted glands failed to survive. We have been unable to consult Walbaum's original paper dealing with cats, and believe Morel has misquoted him, because upon consulting the reference cited by the French author we found that the paper was concerned not with cats, but with rabbits.

Cristiani ('05), who has had very great experience with parathyroid transplantations, states, that notwithstanding central necrosis of the grafts, he was able to demonstrate normal parathyroid tissue five years after autoplastic transplantation in cats.

Biedl ('08) has reported successful parathyroid transplantation in cats and dogs. He implanted the glands into the spleen and asserts that after autotransplantation the removal of the thyroid gland with all the adjacent parathyroids could be borne by these animals without fatal consequences. Biedl further asserted that in some dogs homoplastic parathyroid transplantation was as successful as autotransplantation and that the animals could be kept alive for months after the graft was made and the thyroparathyroid apparatus removed.

Farner and Klinger ('20) performed several parathyroid transplantations in the course of their study of experimental tetany in the cat, and state that "Implantation von Epithel Körperchen anderer Katzen ergab zweimal eine deutliche, aber nur kurz anhaltende Besserung." According to these investigators, the grafts soon underwent autolysis.

For further references to parathyroid transplantation in dogs, rats, and rabbits the reader is referred to the papers of Halsted ('08) and Landois ('11), especially the latter, which has an extensive bibliography.

METHODS EMPLOYED

In our experiments only the two external parathyroids were used for transplantation, the reason being that the internals are very small and variable in position in the cat. They are difficult to dissect free from thyroid and their own investing membranes with speed and accuracy without injury to the glandular tissue. The external glands present none of these difficulties and their size renders them much easier to handle.

After first preparing the area into which the glands were to be transplanted by shaving away the hair and sterilizing the skin, the thyro-parathyroid apparatus and the cervical thymus were removed. The glands were immediately placed in watch crystals containing equal amounts of warm sterile Ringer's solution and blood serum from the animal from which the glands were removed. The parathyroids were dissected from the thyroid lobes, and from their membranes, under a binocular microscope, and immediately transplanted into the area prepared for them. The neck wound in the meantime was packed or covered with sterile gauze and left until after the transplantation was completed in those cases where the graft was made into areas other than the sternohyoid muscles.

The great majority of the homoplastic transplants were made into a pocket cut in the rectus abdominis muscle or else the tissue was placed between the peritoneum and the fascia of this muscle. All bleeding into the wound was avoided, because of the danger of the clot's smothering the graft, and when bleeding occurred after the muscle was incised, another incision was made near by. It is important to mark the exact location of the transplant by a stitch immediately above the position of the implanted tissue, because of the difficulty of locating the small grafts in case their reextirpation is desired. It has been our experience with autoplastic transplantation that the grafts will grow equally well when placed in the subcutaneous tissues as in the rectus abdominis or sternohyoid muscles, but it is exceedingly difficult to locate their

exact position when the wound has closed and healing is complete. There is another reason why the muscle is preferable to subcutaneous tissue: Sometimes small stitch infections may occur and contaminate the surrounding area including the transplanted tissue, thus destroying the graft. Such infections are not common and are never severe, but we have had several subcutaneous grafts vitiated in this way. Autopsy has shown in each case that the area of infection had not spread to the muscle, and we have not observed in our material any traces of infection of the graft area when the transplant was made deep in the muscle tissue.

The major part of our transplants were made within ten or fifteen minutes after removal of the thyro-parathyroid apparatus from the donor. It was our impression after surveying the literature that ten or fifteen minutes was about the maximum time that parathyroid tissue would survive outside of the body. Such is not invariably true, however. In several of our cases we have kept the tissue forty-five minutes in the warm serum-Ringer solution and obtained successful growth following autoplastic implantation, as attested later by reextirpation of the graft and histological examination.

Besides transplantation in or behind the rectus abdominis muscle, other places were tried, such as the vascular area of the ear, the sternohyoid muscle, and the spleen. In our experience the spleen is not a good place in which to graft parathyroids; first, it is too vascular in the sense that the slightest cut induces marked oozing and consequent danger of smothering the implanted tissue in a clot, and, secondly, an abdominal operation is required in order to reach the spleen. In any event, considering our success with autoplastic grafting both with cats and dogs, the spleen cannot be very much more favorable than the rectus abdominis muscle, and in our experience it is much less so.

Following the operation, the animals were fed milk during the first twenty-four hours, and after that time, raw liver or beef with an occasional dish of milk.

We take this opportunity to express our obligation to Mr. Kenneth Donahue, technical assistant in the Osborn Laboratory, for his valuable assistance in administering the anesthetic and for his care of the operated animals.

AUTOPLASTIC TRANSPLANTATION

Six cats were autoplastically transplanted. In four animals the parathyroids were implanted into a pocket cut in the sternohyoid muscle at the time of operation; the remaining two were transplanted in the rectus abdominis muscle. Our original intention was to perform a large series of autoplastic transplantations, to determine, if possible, the length of time the parathyroids could be left outside of the body in the blood-serum-Ringer mixture and survive when grafted. Cats, however, proved to be unsatisfactory material for this sort of experiment, because large numbers of these animals possess accessory parathyroid glands and do not develop tetany when the usual four glands are removed. Halsted ('08) and others have stated that, in order to obtain successful autoplastic parathyroid grafts, it is first necessary to create a parathyroid deficiency greater than 50 per cent. That is to say, a physiological deficit is created and the graft is more likely to 'take' under such conditions than when there is no functional need on the part of the organism for the glandular tissue. Our experience with parathyroid extirpation in cats ('23, '24) has shown that removal of four parathyroids leads to no untoward symptoms in large numbers of these animals, and we were able to demonstrate that presence of considerable accessory glandular tissue in these cases—amounts sufficient to suffice for the needs of the organism. However, in cats, removal of four parathyroids does not necessarily create a physiological deficit; one small parathyroid is amply sufficient to maintain a cat in a normal healthy condition. Thus it is obvious, that where cats are used as experimental material, one can never be quite certain of fulfilling the conditions laid down by Halsted as necessary in order for autoplastic parathyroid grafts to survive and func-

tion. Because of these facts, this phase of the problem was turned over to one of the graduate students working with the senior writer (W. W. S.) to work out on dogs. A detailed account of this work will appear later; suffice it to say here that the work on dogs confirms in every way the data obtained by us on cats in regard to the success of autoplastic grafting.

Our experimental material is extensive and the history of each individual case cannot be given in detail. The following abbreviated protocols represent typical cases occurring in our experiments.

Cat 1. October 10th. Adult female; weight, 2470 grams. Thyroparathyroid apparatus removed and also the cervical thymus. All four parathyroids identified. Both external parathyroids, dissected free of their connective-tissue investure, were implanted into a pocket cut in the rectus abdominus muscle. Glands out of body twelve minutes.

October 11th. No symptoms. Cat eats heartily.

October 12th. Slight fibrillary tremors of flank and hind-leg muscles present. Typical paw shaking. Tetany eye sign present. Cat refuses food.

October 13th. Symptoms same as above, except tremors are more obvious.

October 14th. Marked tetany, animal prostrate. Extensor spasms of the limbs, muscular twitchings and tremors present. Animal refuses food.

October 15th. Animal better. Tetany abating. Tremors and twitchings not so marked. Animal walks about. Cerebellar symptoms present. Drinks milk.

October 16th. No change. Animal takes food. Lies quiet in cage and sleeps most of the time. Weight, 2120 grams.

October 17th–18th. No tetany symptoms except for slight stiffness in limbs when walking. Tetany eye sign has disappeared.

October 18th–25th. No symptoms, animal apparently completely recovered. The neck wound has healed. Animal eats meat in large quantity. Is playful and appears perfectly normal.

November 15th. Graft area extirpated. Weight of cat, 2455 grams.

November 16th–17th. Animal shows tetany symptoms. Tetany eye sign present; fine tremors of head and flank muscles noticeable. Cat refuses food.

November 18th–19th. Animal prostrate and is in violent tetany. Epileptiform convulsions ending in rigidity and unconsciousness occurred twice.

November 20th. Animal died in convulsions. Autopsy showed no trace of thyroid or parathyroid apparatus. A small blood clot was present on an area from which the graft had been removed. Weight of animal, 2302 grams.

Cat 2. October 16th. Adult male; weight, 2640 grams. Thyro-parathyroid apparatus and cervical thymus extirpated. Four parathyroids identified, and the two externals transplanted into the sternohyoid muscle. Glands out of body fourteen minutes.

October 17th. Cat depressed; refuses food. Eye sign apparent. No tremors noted.

October 18th. Tremors present, also paw shaking and cerebellar symptoms. Tetany eye sign marked. Animal depressed.

October 19th. Tremors barely visible. Eye sign present and occasional paw shaking. Animal drinks milk. Weight, 2562 grams.

October 20th. Tetany eye sign present, but no other symptoms. Animal eats heartily. From this date on until November 18th, the cat showed no further signs of tetany. The neck wound healed per primum. Animal fed milk and liver each day.

November 18th. Graft area removed. Weight of cat, 2627 grams.

November 19th–24th. Tetany symptoms first noted on evening of 19th and on morning of 20th. The animal was in violent tetany. This continued for several days, the animal finally dying in convulsions on November 24th. Weight, 2421 grams. Autopsy of the neck region failed to reveal any thyroid or parathyroid tissue.

Cat 3. October 16th. Half-grown male; weight, 1436 grams. Operation same as described for cats 1 and 2. Two external parathyroids transplanted into sternohyoid muscle. Glands out of body seventeen minutes.

October 17th–November 16th. Animal showed no symptoms whatever. On 16th of November the graft area was removed and the animal was observed for a week, but no symptoms developed. The animal was then killed and a careful examination of the neck region undertaken. A suspicious piece of tissue was found embedded in the posterior portion of the cervical thymic lobe which, upon histological examination, proved to be an accessory parathyroid glandule. There can be little doubt that the failure of this animal to develop tetany following reextirpation of the graft was due to this accessory tissue.

Cat 4. October 22nd. Adult female; pregnant; weight, 3021 grams. Thyro-parathyroid apparatus and cervical thymus removed. The external parathyroids were implanted into sternohyoid muscle. The glands were out of the body twelve minutes.

October 23rd–24th. Cat has tetany symptoms, shakes paws, eye sign present, has tremors of head and body muscles. Depressed and refuses food.

October 24th–26th. Animal seems better. Symptoms abating. Cat eats meat. Weight, 3000 grams.

October 27th–November 28th. Animal completely recovered. Showed no tetany symptoms. Slight infection of neck wound developed, but was controlled by irrigation with Dakin's fluid.

November 28th. Graft area reextirpated. Weight of cat, 3440 grams.

November 29th–30th. Tetany symptoms developed, such as tremors, paw shaking, and the eye sign, but none were marked. Cat seemed depressed and refused food.

November 30th–December 3rd. Symptoms same as before. Animal presents all the indications of latent tetany and, in fact, can be thrown into active tetany by rattling the cage or frightening the animal. The induced tetany lasts but an hour or two and then disappears. This condition persisted for ten days, the cat finally recovering completely.

December 16th. Cat chloroformed and the neck region carefully examined. All suspicious-looking tissue was examined under the binocular microscope. The thoracic thymus was likewise examined, but accessory parathyroids were not found. The thymus tissue has not yet been sectioned. We believe that the cat had minute accessory parathyroids present too small to be detected except by serial sections of the entire neck region (a method obviously impossible to us in so large an animal as the cat) and that these glandules were barely sufficient to work off tetany symptoms following reextirpation of the graft. The next case is of considerable interest in this connection.

Cat 5. November 10th. Female, adult; weight, 1801 grams. Operation as described above. Two external parathyroids transplanted into a pocket in the rectus abdominis muscle. Glands out of body eighteen minutes.

November 11th–15th. Cat showed tetany symptoms, but they were not marked. Tremors, paw shaking, depression, loss of appetite, and the eye sign were all present. Weight, 1764 grams.

November 16th–30th. Cat recovered completely and appeared perfectly normal. Developed a severe conjunctivitis, but this was cured by continuous treatment with a 1-2000 solution of silver nitrate.

December 14th. Graft area removed. Weight of cat, 1820 grams.

December 15th–24th. Animal exhibited the typical symptoms of chronic tetany in which typical tetany symptoms were present, including intervals of marked depression. On the 24th the cat was killed and autopsied. Nothing even remotely resembling accessory parathyroids was found. The upper portion of the thoracic thymus

was sectioned and two very small nodules or rather clusters of undoubted parathyroid cells were found. These accessory glands were the smallest the writers have yet observed and could not possibly have been detected by macroscopic examination. They were *probably* just able to function sufficiently to ward off fatal tetany—we say *probably* advisedly, because the amount of parathyroid tissue just sufficient to keep an animal alive is unknown.

Cat 6. December 4th. Adult male. Weight, 2241 grams. Operation as previously described. The two external parathyroids were implanted into a pocket cut in the sternohyoid muscle.

December 4th–January 3rd. No symptoms ever developed. On January 3rd, the graft area was removed and the animal kept for ten days under observation, but tetany symptoms did not develop. The animal was killed January 10th by injections of guanidine sulphate. Autopsy revealed no evidence of parathyroid tissue. The thoracic thymus was preserved for sectioning, but was never used, owing to the fact that serial sections of the graft area made some time later showed that a part of one of the grafted glands had been left in the animal, so that the reextirpation was incomplete. The fact that the animal never developed tetany symptoms following both operations strongly hinted at the presence of accessory glands. As the carcass was not preserved, we have no means of knowing one way or the other.

The graft tissue was preserved in Zenker's fluid and, when sectioned, stained in Delafield's haematoxylin and Congo red. Sections were cut at a thickness of 8 μ. Although both external parathyroids were grafted together, generally only one gland or a part of it survived in good condition. This was found to be the case in cats 1, 3, 4, and 5. Case 2 showed both glands persisting, though normal functional parathyroid tissue was observed only at the periphery of each gland. The graft could not be located in cat 6. Thus the evidence indicates that the peripheral portions of two grafted parathyroids are capable of surviving side by side in cats, and we have two cases of two autoplastically implanted parathyroids of dogs which survived. This case will be reported elsewhere.

Time and space can best be served by presenting the data obtained by histological study of the autoplastically transplanted glands together, because the histological data are very much the same in all cases.

The parathyroid grafts are encapsulated in a connective-tissue sheath which may be of varying thickness, and through which the blood vessels pass to the central area of the tissue

(fig. 1). The peripheral edges of the grafts are almost invariably in excellent condition and are quite evidently normal healthy tissue. The thickness of this periphery of normal parathyroid tissue varies in the individual cases, sometimes being quite thick throughout the circumference or thick along one border of the graft and consisting of rather thin and much broken-up areas along the other. In some cases the peripheral border of normal tissue is very thin. Various regions of each individual transplant show considerable variations of this nature. The cells appear perfectly normal and cannot be distinguished from those which make up any normal parathyroid.

The central area of each gland is invariably necrosed and has been replaced by connective tissue (figs. 1 and 6). In those cases where the graft had been left in the animal for considerable time, no vestige of parathyroid cells can be detected in the entire central area. This space also contains numerous blood vessels. Figure 1 represents a section of a gland which had been transplanted into the rectus abdominis muscle thirty-six days. The section is typical of the structure of such autoplastic parathyroid grafts. None of the grafts in our cases were older than thirty-seven days, and it is possible, though we do not regard it as very probable, that the glands might later have undergone complete resorption.

Cristiani ('05) states that after the lapse of five years he was able to demonstrate normal parathyroid tissue in an autotransplant of a parathyroid in a cat.

We have noted in normal parathyroids of cats that in some glands the central area may be largely composed of connective tissue carrying blood vessels, and large lymph spaces. Such a gland is figured in our earlier paper (fig. 4), "An experimental and morphological study of the parathyroid glands of the cat," which appeared in this journal a few months ago. Other glands from other individuals may or may not show a comparable central area of the type figured.

The histological structure of the grafted glands of cases 3, 4, and 5 is of especial interest, because all of these cases failed

to develop fatal tetany or depression following reextirpation of the grafts. The evidence is clear that the reason for this seemingly anomalous situation is due to the presence of accessory parathyroid tissue in these animals. This was certainly true of cases 3 and 5, where sections of the thymus revealed the presence of accessory parathyroid tissue. How-

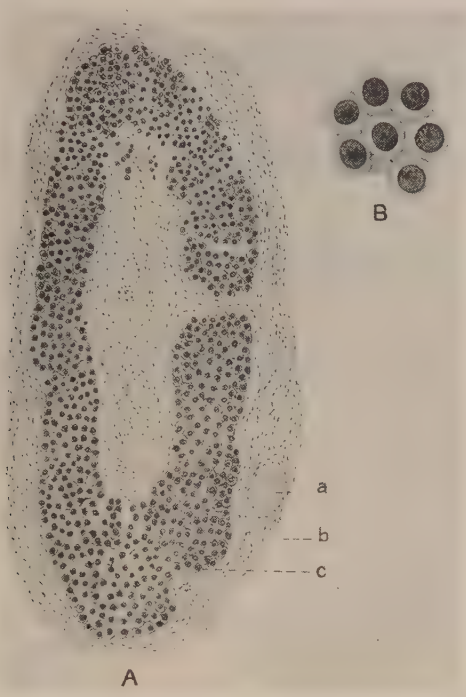

Fig. 1 A. Sketch of an autoplastically transplanted parathyroid thirty-six days after implantation. *a*, blood vessel in *b*, the connective-tissue sheath, in which the gland *c* is embedded. B. High-power view of the parathyroid cells composing the gland. × 900.

ever, the grafted glands of these individuals differed in no way from those of the cases which ended fatally following removal of the graft and in which accessory tissue was evidently not present, or at any rate present in amounts insufficient to preserve the life of the individual (fig. 5). Several authors, notably Halsted ('08), have stated that one condi-

tion necessary for the survival of autoplastic grafts of the parathyroids is the creation of an anatomical deficit of parathyroid tissue of at least 50 per cent. In all of our cases this condition was of course complied with, for it is extremely

TABLE 2

Autoplastic transplantation

CAT	LOCATION OF GRAFT	SYMPTOMS FOLLOWING THYROPARATHYROIDECTOMY	RECOVERY	REEXTIRPATION OF GRAFT	RESULT	REMARKS
1	Rectus abdominis muscle	Tetany	Complete	36 days after implantation	Fatal tetany	Graft in good condition. Central area replaced by connective tissue
2	Sternohyoid muscle	Tetany	Complete	33 days after implantation	Fatal tetany	Graft in good condition. Both glands healed in. Central area replaced by connective tissue.
3	Sternohyoid muscle	None	Complete	31 days after implantation	No symptoms	Graft in good condition. Central area replaced by connective tissue. Accessory glands found at autopsy
4	Sternohyoid muscle	Tetany	Complete	37 days after implantation	No symptoms	Graft in good condition. Central area replaced by connective tissue. Accessory parathyroids probably present
5	Rectus abdominis muscle	Tetany	Complete	34 days after implantation	Chronic tetany	Graft tissue well preserved. Central area as in other cases. Small accessory parathyroids found in thymus
6	Sternohyoid muscle	None	Complete	30 days after implantation. Glands not found	No symptoms	Graft not located

doubtful if any of the animals possessing accessory parathyroid material had it in amounts equal to one-half of the amount removed at the time of operation. An anatomical deficit of 50 per cent of the normal does not necessarily imply a physiological deficit of the same degree, for it is surprising what a minute amount of parathyroid tissue is sufficient to

maintain a cat or a dog in normal healthy condition. Table 2 summarizes the results of our experiments on autoplastic transplantations.

HOMOPLASTIC TRANSPLANTATION

The majority of the transplantation experiments were of this type, twenty-eight animals being used. In all cases the cats were of the same sex and as nearly as possible of the same age as judged by their size. The animals were obtained from various sources, and it was impossible to obtain any information as to their exact age. It is unfortunate that animals belonging to the same family, i.e., brother or sister cats, could not have been obtained in sufficient numbers for the experiment. At present we have on hand fourteen kittens of known age and parentage, so that in the near future homoplastic transplantation of the parathyroids into individuals of the same sex and parentage can be carried out.

The homoplastic transplantations were of two kinds: 1) where the grafts were made immediately following the onset of tetany symptoms (and by tetany symptoms we also mean depression, which is as characteristic of parathyroid removal as the violent neuromuscular phenomena), and, 2), simultaneous or crisscross grafting, in which case the parathyroids of cat A were transplanted to cat B and vice versa. This procedure of course necessitates two simultaneous thyroparathyroidectomies.

A. Transplantation following the onset of tetany symptoms

1. Grafts into the rectus abdominis muscle. Most of the homoplastic transplantations were made into a pocket cut into the rectus abdominis muscle or the tissue was placed beneath the muscle, between the fascia and the peritoneum. Halsted ('08) recommends this site for grafting, and in our experience it is far preferable to the spleen or peritoneum as the position of choice for parathyroid transplantation. Six animals were transplanted in this manner following the onset of tetany symptoms. Several cats showed

considerable improvement following transplantation and two completely recovered.

Cat 64. January 28th. Female; weight, 2935 grams. Thyro-parathyroidectomy.

January 29th. Cat normal and shows no symptoms.

January 30th. 9:00 A.M. Animal showed slight tremor of lower jaw; salivation and tetany eye sign. At 2:00 P.M., marked muscular tremors of the head region and salivation were noted. At 5:00 P.M., two external parathyroids transplanted.

January 31st. Cat has violent tetany. Injected with 10 cc. calcium lactate. Tetany disappeared after two hours, only to return again later.

February 1st–3rd. Animal has bad tetany. Given repeated injections of calcium lactate.

February 3rd–8th. The symptoms are not so marked. The animal is very weak and must be forcibly fed.

February 9th. Animal found dead in cage. Weight, 2250 grams. Autopsy revealed no trace of thyroid or parathyroid tissue present in neck region.

This animal lived ten days after transplantation, and throughout this period was never free from tetany symptoms. It is questionable whether the graft really helped the cat and postponed the fatal issue or whether it was not the calcium injections which tided the animal over a few days. The animal lived an unusual time, considering the severity of the tetany exhibited.

Histological examination of the graft (fig. 2) showed that the entire area had undergone extensive necrosis. Only a narrow rim of tissue at the periphery preserved any resemblance to a normal appearance. In the necrosed area the cellular elements were barely discernible as separate entities. The nucleus and cytoplasm of the cells formed a single undifferentiated mass. Cell outlines were obliterated, the whole area staining a pinkish brown. This central mass was dotted with deep blue staining connective-tissue nuclei migrating in from the periphery, and contained numerous capillary vessels ramifying throughout.

The peripheral border of cells is very sharply demarkated from the central necrotic area, both in histological structure

and staining reaction. The peripheral cells present many normal features. The nuclei are stained deep blue, the cytoplasm a light yellowish brown. The nuclei appear normal, but the cell cytoplasm is vacuolated and cell borders are vague and the cells not sharply marked off from one another as in normal parathyroid tissue and autoplastic grafts. Scattered

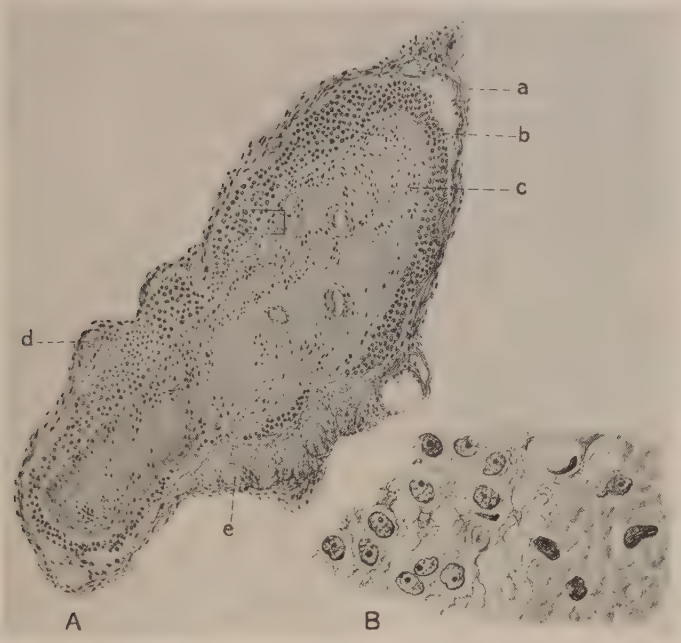

Fig. 2 Cross-section of a homoplastic parathyroid graft. *a*, connective-tissue sheath; *b*, peripheral ring of slowly degenerating parathyroid cells; *c*, necrotic central area of the graft; *d*, blood vessel; *e*, masses of blood cells. B. High-power magnification of the area marked out in A.

here and there are clusters of cells which to all appearances are normal, and if seen in sections of non-grafted material would not attract attention as being abnormal. For the most part, however, the entire peripheral area presents the appearance typical of homoplastically transplanted parathyroid tissue, i.e., hazy and vacuolated appearance of the cytoplasm, vagueness of cell boundaries, and crinkling of the limiting

membranes of the cells. Figure 2 is a section through the graft and illustrates some of the conditions described.

The graft was well vascularized, the implanted tissue being surrounded by a fine mesh-work of capillaries; sections show that the vessels penetrated into the central area of the graft. Along one edge of the graft was a mass of blood cells, many also penetrating into the graft; at this point the rim of persisting parathyroid cells was obliterated. The transplant was limited from the surrounding muscle tissue by a definite connective-tissue investure through which run the blood vessels.

The history of the next case, cat 66, is of interest, because this is one of the few cases where a homoplastic graft 'took,' at any rate in the sense that it retained a more or less normal histological structure for a considerable period. The case is also of interest because of the numerous experiments performed on this animal. Whether or not the various experimental conditions to which the cat was subjected had anything to do with the preservation of part of the graft is not known; it is possible, but does not seem very probable, in view of somewhat similar findings on other cats not so treated.

Cat 66. February 2nd. Male; weight, 2010 grams. Thyro-parathyroidectomy.

February 3rd. No symptoms in A.M. 8:00 P.M., fine tremors of head muscles apparent; tetany eye sign present.

February 4th. Cat has fibrillar twitchings of flank and head muscles. Marked spasticity of hind legs. Transplanted at 2:00 P.M.

February 5th. No tremors present. Tetany eye sign present, and animal seems depressed.

February 6th and 7th. Animal depressed; refuses food. No tremors noted.

February 8th. Cat better, drinks milk and walks about.

February 9th–10th. Cat apparently normal. Eats meat and seems in good condition.

February 10th–14th. Cat normal. No symptoms.

February 14th–16th. No symptoms.

February 17th. Animal injected subcutaneously with 200 mg. of methyl guanidine sulphate. Tremors, salivation, and restlessness resulted. Animal recovered completely three hours later.

February 18th. Cat shows no effects of guanidine injection.

February 21st. Animal given to one of the graduate students for operation upon the adrenals. Both adrenals removed soon afterward. Animal died March 3rd of adrenal insufficiency. The cat died in convulsions, with rigidity, and opisthotonus. Autopsy showed no trace of thyroids, parathyroids, or adrenals present. The thoracic thymus was not sectioned, and it seems probable that some minute masses of parathyroid tissue were present there. The grafted tissue was well healed in and encapulated in a connective-tissue sheath (fig. 7).

Histological examination of the graft shows that part of it had preserved its integrity in the foreign environment. Portions of both external parathyroids persisted in fairly good condition side by side. This is unusual because generally, though not invariably, only one grafted gland will persist in good condition even in autoplastic grafts. The grafted tissue was deeply embedded within the muscle tissue (fig. 7).

The central areas of the glands were not greatly necrosed—not, at any rate, as extensively as one might expect to be the case. The vascularization of both glands was excellent, numerous small vessels penetrating deep into the central area. When examined hastily, the glands appear to be normal, but careful examination reveals that the parathyroid cells are slightly irregular in outline, with a somewhat vacuolated cytoplasm. However, part of this transplant presented an appearance very close to normal, despite the fact it had lived in the foreign environment for thirty days. The question whether or not this graft was functional cannot be definitely answered one way or the other, except to call attention to the fact that the animal developed tetany symptoms following operation and recovered completely after transplantation. It is unfortunate that the graft area was not later reextirpated, thus testing its physiological efficiency. Table 3 summarizes the main points of this series of experiments.

2. Grafts into the vascular area of the ear. Years ago, R. Cristiani called attention to the fact that in rats parathyroid transplantation into the ear could be done successfully, and because of the ease with which the operation could be per-

formed and the fact that the graft could be observed at any time without the necessity of an operation, he recommended this region as a good place for gland implantation. Camus ('05) has also employed the ear for parathyroid transplantation. He used rabbits, but was unable to obtain good results. In a later paper ('05), evidently stimulated by the negative

TABLE 3

Grafts into the rectus abdominis muscle after onset of tetany

CAT	SEX	WEIGHT	SYMPTOMS AFTER THYRO-PARATHYROIDECTOMY	GRAFTED	SYMPTOMS	RESULT	DURATION OF EXPERIMENT AFTER GRAFTING	WEIGHT	REMARKS
		grams					*days*	*grams*	
50	♂	320	Tetany	24 hours after operation	Tetany and depression	Death	3	290	Degeneration of the graft
52	♀	420	Tetany	24 hours after operation	Tetany	Death	2	408	Graft not sectioned
64	♀	2935	Tetany	2 days after operation	Tetany	Death	10	2250	Degeneration of graft
65	♂	2010	Tetany	24 hours after operation	Tetany	Death	4	1800	Degeneration of graft
66	♂	2001	Tetany	2 days after operation	Slight tetany	Recovery	32[1]	1979	Part of graft persisted in fair condition.
68	♂	3660	Tetany	2 days after operation	Tetany and depression	Recovery	36[1]	3400	Degeneration of graft. Accessory parathyroids probably present

[1]Animal succumbed to adrenal insufficiency due to removal of both adrenals. This accounts for the weight loss.

findings of Camus, Cristiani called attention to the fact that ear transplantations in rabbits would be much less likely to survive them in rats, because of the large amount of cartilage present in the rabbit ear which would be likely to exert pressure effects on the implanted tissue. It is evident, too, that Cristiani employed autoplastic grafts, while Camus used the homoplastic method. The difference in the nature of the

grafts employed by these two investigators is sufficient to account for the discrepancy in their results.

Homoplastic transplantation to the vascular area of the ear was employed in six cases; the transplants consisted of two external parathyroids secured from animals which showed the onset of tetany. Injections of calcium lactate were given repeatedly to the animals when they developed violent symptoms, in order to alleviate the symptoms and give the animal sufficient time for the grafts to heal in and function. Two typical protocols of this series of animals are given below in greatly abbreviated form.

Cat 37. October 6th. Adult female; weight, 2250 grams. Thyroparathyroidectomy.

October 7th. Animal stiff in legs. Slight cerebellar symptoms and paw shaking noted.

October 8th. Cat markedly depressed. Refuses food. Tetany eye sign present. Muscular tremors and labored breathing in A.M. At 4 P.M., cat prostrate. Violent tetany. 16 cc. of 5 per cent solution calcium lactate injected subcutaneously. Cat much improved at 6 P.M., and walks about, though unsteadily.

October 9th. Cat prostrate, but not so bad as on previous afternoon.

October 10th. Two external parathyroids of a female cat implanted into right ear. Also 10 cc. of 5 per cent solution calcium lactate injected subcutaneously. Cat very much improved at 5 P.M. Weight, 1820 grams.

October 11th. Cat markedly improved. Walks about cage and takes some interest in its surroundings. Tremors and cerebellar symptoms marked.

October 12th. Animal weak, but is able to walk about. It cannot eat or drink and must be forcibly fed milk. Trismus marked.

October 13th–14th. Cat feeble and the tetany symptoms have returned. Calcium injected each day. Cat had one epileptiform seizure at 4 P.M., characterized by rigidity and unconsciousness.

October 15th. Cat prostrate. Trismus marked. Continuous convulsions. Repeatedly injected with calcium, but animal steadily grows worse.

October 16th. Cat found dead. Autopsy showed no trace of parathymus in the thyroid region. Weight, 1630 grams. The two grafted parathyroids were very necrotic and had very poor vascularization. Microscopic examination of the grafts showed great degeneration changes in the glands. Few, if any, normal parathyroid cells were present.

Cat 41. October 12th. Adult male; weight, 2600 grams. Thyroparathyroidectomy.

October 13th. No symptoms.

October 14th. Animal depressed. Refuses food. Tetany eye sign present. Slight tremors.

October 15th. Animal spastic in limbs and exhibits slight tremors and depression.

October 16th–17th. Cat greatly depressed. Few tremors present; refuses food. Anorexia and vomiting noted.

TABLE 4

Grafts of the parathyroid into the ear following the onset of tetany

CAT	SEX	WEIGHT	SYMPTOMS AFTER THYRO-PARATHYROIDECTOMY	GRAFTED	SYMPTOMS	RESULT	DURATION OF EXPERIMENT AFTER GRAFTING	WEIGHT	REMARKS
		grams					*days*	*grams*	
34	♀	550	Tetany and depression	5th day after operation	Tetany and depression	Death	4	450	Graft degenerate. Little vascular connection
35	♀	820	Tetany and depression	3rd day after operation	Tetany and depression	Death	4	560	Degeneration of graft. Poor vascularization
37	♀	2250	Tetany	4th day after operation	Tetany	Death	6	1630	Graft degenerate
38	♀	600	Tetany and depression	4th day after grafting	Tetany and depression	Death	5	502	Graft degenerate
41	♂	2600	Tetany	7th day after operation	Tetany	Death	6	1000	Graft degenerate
42	♂	1801	Tetany	2 days after operation	Tetany	Death	4	1740	Graft degenerate

October 18th. Fine tremors head and trunk muscles are present. Animal is spastic, refuses food, and exhibits cerebellar symptoms when walking. Animal forcibly fed milk.

October 19th. Paw shaking and marked muscular tremors noted. Cat must be fed forcibly. Trismus present. 11 A.M., three parathyroids of male cat transplanted to right ear; two external and one internal gland were used.

October 19th–22nd. Animal seemed greatly improved. Tremors, cerebellar symptoms, and tetany eye sign present. Forcibly fed milk.

October 23rd. Cat very weak, but walks about; exhibits marked fibrillary tremors of head and shoulder muscles. Cat greatly emaciated.

October 24th. Animal found dead. Autopsy showed no accessory parathyroid tissue. Weight, 1000 grams. Examination of the grafted tissue showed one gland to be almost completely degenerated. The remaining gland had undergone great degeneration, but at periphery of graft a thin border of undegenerated tissue remained. This tissue, however, was not normal, the cells were vacuolated and shrunken, the cells were irregular in outline and difficult to make out clearly The graft area appeared to be poorly vascularized.

The two animals just described both lived sufficiently long for the implanted tissue to have time to 'take' and exert some effect. They are typical of this series of ear transplantations. A detailed description of the remainder of the cases would be of little value. Table 4 summarizes the findings.

B. Transplantation before the onset of tetany

This type of experiment is superior to the other method employed for determining the functional significance of homoplastic parathyroid grafts. It is far more difficult to cure tetany once it has developed than it is to prevent its occurrence. This fact has been brought home to the senior writer (W. W. S.) by observations upon dogs which had been autoplastically grafted and upon parathyroidectomized dogs on tetany preventing diets. Once such animals have developed tetany by reason of changes in the diet or because of an infection, which predisposes operated animals to tetany, it is difficult, indeed, to cure the animals. The same is true of cats, though not to the same degree, because in these animals the onset of tetany is generally not so violent as it is in dogs.

Then, too, animals grafted after the onset of tetany generally succumb before the graft has had sufficient time to properly function. In our series of animals transplanted after the onset of tetany none survived, whereas in the series of sixteen animals transplanted simultaneously with removal of the thyro-parathyroid apparatus, one-half survived. At least one-third of the animals which survived would doubtless

have done so even without transplantation, because of the presence of accessory parathyroid glands. But even though one-half of the surviving animals be ruled out because of this fact, the per cent of survivals and recoveries following crisscross operation is unusually high. Our data indicate that this method of transplantation probably tides the organism over a critical period of two or three days following removal of the parathyroid apparatus, thus giving the accessory parathyroid glands (present in at least 35 per cent of cats as revealed at autopsy and probably present in a much higher per cent) opportunity to take over the functional activity of the parathyroid apparatus. We shall return to this question later and discuss it more in detail, after first presenting some of the evidence for our conclusions.

Table 5 summarizes briefly the principal data of the experiment. A few typical protocols are given below in abbreviated form.

Cat 74. March 19th. Adult female; weight, 2700 grams. Thyroparathyroidectomy. Two external parathyroids of another female animal transplanted beneath the rectus abdominis muscle between the peritoneum and the fascia.

March 20th. Cat exhibits tetany symptoms, such as paw shaking, spasticity, and the tetany eye sign.

March 21st. Symptoms same as before.

March 22nd. Violent tetany, constant tremors of head and flank muscles. Extensor spasms of the legs very pronounced. One epileptiform convulsion occurred, ending in rigidity and unconsciousness.

March 23rd. Animal is prostrate and in continuous convulsions. 12 cc. of a 10 per cent solution of calcium lactate was injected. Cat was much better one hour later.

March 24th. Cat appears much better, but is greatly depressed. Eye sign is very pronounced. Animal refuses food. Vomits when forcibly fed milk.

March 25th. Cat greatly depressed, but has no tremors. Eye sign present. The animal is so weak it walks unsteadily. Death occurred at 5 P.M. Autopsy showed the graft to be well healed in and well vascularized. Weight, 2100 grams. Figure 3 shows the microscopic appearance of the graft.

Histological examination of the graft (fig. 3 showed it to be well healed in and firmly adherent to the surrounding

TABLE 5

Transplantation of parathyroids into the rectus abdominis muscle before the onset of tetany

CAT	SEX	WEIGHT	SYMPTOMS	RESULT	DURATION OF EXPERIMENT	WEIGHT	REMARKS
		grams			*days*	*grams*	
53	♂	650	Depression	Death	3	600	Graft poorly vascularized. Not sectioned
54	♀	1510	Tetany	Death	7	1205	Degeneration of the grafted glands
55	♀	2420	Slight tetany	Recovery[1]	Graft removed after 21 days. No symptoms	2020	Parts of grafted glands persisted in fair condition. Accessory parathyroids probably present
56	♂	5050	No symptoms	Recovery	22	4786	Degeneration of graft
57	♂	3240	Tetany	Recovery	20	3915	Degeneration of graft. Small accessory parathyroid found in thoracic thymus
58	♂	3011	No symptoms	Recovery	Graft removed after 10 days. No symptoms	3024	Degeneration of graft. Peripheral border in fair condition
59	♂	3565	Tetany	Death	7	3430	Degeneration of graft
60	♂	1210	No symptoms	Recovery	12	1314	Degeneration of graft. Accessory parathyroid found in thymus
61	♂	2540	Tetany	Recovery	30	2601	Degeneration of graft. Accessory parathyroid tissue not found
69	♀	3160	No symptoms	Recovery	30	2540	Parts of both grafted glands persisted in fair condition
70	♂	3120	Tetany	Death	3	3020	Graft not sectioned
71	♂	2750	Tetany	Death	4	2610	Degeneration of entire graft. Gland area well vascularized
72	♀	3420	Tetany	Death	4	3360	Grafts made subcutaneously on account of pregnancy. Could not locate the grafts later
73	♀	1824	Tetany	Death	4	1773	Great degeneration of the grafted tissue. Glands well vascularized
74	♀	2700	Tetany	Death	7	2100	Degeneration of central area of graft. Peripheral area persisted in fair condition
75	♀	2250	No symptoms	Recovery	45	2478	Parts of both glands in fair condition. Accessory parathyoid present

[1] It is probable that most of the cats which recovered had accessory parathyroids.

muscle. The central area of the graft had undergone degeneration and had lost all resemblance to parathyroid tissue. The individual cells are vague and indistinct in outline and the nuclei are not well marked off from the cytoplasm. Many deeply staining nuclei appear scattered throughout the mass of necrotic parathyroid cells; they stand out clearly and in striking contrast to the degenerate mass in which they lie. They appear to be connective-tissue elements migrating in from the peripheral region of the graft.

The outer edge of the graft shows the usual narrow rim of undegenerated parathyroid cells, i.e., the degenerative changes had not progressed to the stage where cell outlines were totally obliterated, although great degenerative changes had occurred. The nuclei of the cells of this region stain dark blue. The cells do not appear normal or healthy; the cytoplasm is shrunken and the cell outlines are not clear-cut and regular. In some places the peripheral rim of cells has completely disappeared.

The graft was exceedingly well vascularized (fig. 3, B). Blood vessels from the surrounding muscle area had grown in and formed a fine network over the graft surface and over the surface of the muscle pocket in which the grafted glands lay. These small blood vessels had penetrated deeply into the substance of the graft ramifying throughout the necrotic central area. Lack of vascular supply certainly was not responsible for destruction of the transplant. In figure 4 is shown a portion of a transverse section through the graft, showing the marked ingrowth of blood vessels. They seem to be as numerous at the center as at the periphery. In normal parathyroids the blood vessels are not nearly so numerous at the periphery of the gland as in this transplant.

Figure 4 shows the macroscopic appearance of a parathyroid graft four days after implantation. Note the vascularization of the parathyroids. Microscopic examination showed marked degenerative changes in progress (cat 71).

The history of our next animal is of great interest because it is one of the very few cases in our experiments in which

portions of the homoplastically transplanted parathyroids persisted more or less unchanged (histologically at any rate) after implantation. Because of the importance of the histological findings, the history of the cat will be given in detail.

Cat 55. November 28th. Female; weight, 2420 grams. Thyroparathyroidectomized and two external parathyroids of another cat

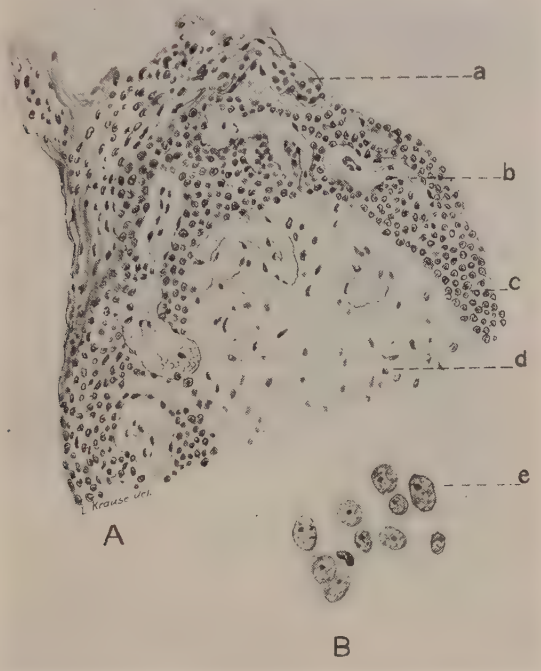

Fig. 3 A. Low-power magnification of a transverse section through part of a homoplastic parathyroid graft. *a*, sheath of connective tissue; *b*, blood vessel; *c*, peripheral ring of degenerating cells; *d*, necrotic central area. B. Higher magnification of the type of cells at the periphery of A. Note that the cells *e* are shrunken and irregular in size and shape.

transplanted under the rectus abdominis muscle between the peritoneum and the fascia.

November 29th. No symptoms aside from slight spasticity. Cat eats heartily.

November 30th. Tetany eye sign present, also slight tremors of head and flank muscles.

December 1st. Animal depressed. Slight fibrillary twitching head and flank muscles. No paw shaking.

December 2nd. Paw shaking observed; animal depressed and refuses food.

December 3rd. Animal seems much better. Eats heartily.

December 4th–19th. Animal appeared normal, but exhibited the tetany eye sign and at times suffered from depression.

December 19th. The graft was removed. Weight of animal, 2020 grams. The graft had healed in perfectly and was well vascularized.

December 19th–25th. No tetany symptoms resulted from removal of the graft. On the 25th the animal was killed by an injection of 610 mg. of methyl guanidine sulphate. Autopsy failed to show any accessory parathyroids, but the thoracic thymus was not sectioned. It seems probable that such glands were present.

Histological examination of the grafted glands revealed the surprising fact that portions of the grafts had retained a normal histological appearance and apparently had 'taken,' or at any rate, parts of the graft had preserved a fairly normal histological appearance. Only small portions of the two grafted parathyroids persisted. The glands showed considerable necrotic changes. The central areas were only slightly invaded by connective tissue, but the edge of one of the grafts had undergone almost complete degeneration. The remainder of the glands was in fair condition and histologically presented the appearance of normal parathyroid tissue. The tissue was broken up into cords of cells separated by fine connective-tissue partitions.

The persistence of portions of this graft and also that of case 66 shows that under certain unknown conditions homoplastic parathyroid grafts can survive in a foreign host or at any rate can preserve their histological structure practically unchanged for considerable periods. Both animals in which portions of the grafts apparently 'took' showed obvious indications of parathyroid insufficiency, such as tremors, the eye sign, and paw shaking. However, neither reacted violently to parathyroid extirpation, the condition being mild and more or less chronic in character. That both animals possessed accessory parathyroids there can be little doubt, though autopsy failed to reveal them. However, in neither case was the thymus sectioned, and our work has shown

clearly that this structure in cats generally contains some accessory parathyroid tissue. The amount present in Cat 55 must have been minute, because the animal showed some evidence of a chronic parathyroid insufficiency throughout the experiment.

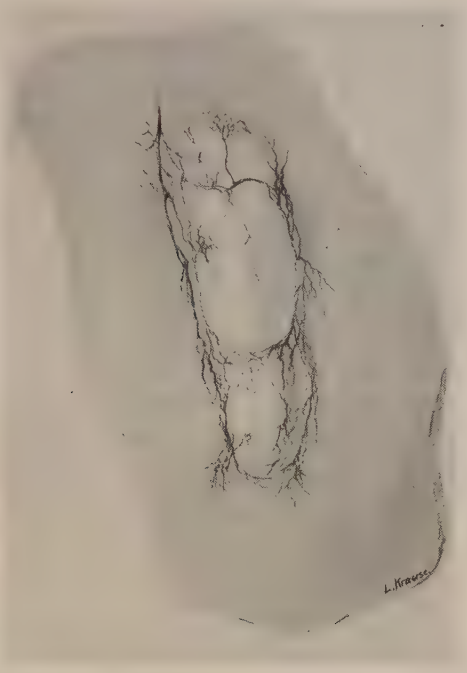

Fig. 4 Macroscopic appearance of a four-day homoplastic parathyroid transplant consisting of an external and internal gland viewed from the peritoneal surface. Note the extensive capillary network.

The large number of animals surviving parathyroidectomy after crisscross transplantation (50 per cent), compared with the almost 100 per cent mortality following transplantation after the onset of the tetany syndrome, seems to indicate that this type of operation, by tiding the organism over a critical period of two or three days immediately following thyroparathyroid removal, gives any accessory glands that may be present an opportunity to become functional.

Our results on cats throw considerable light upon the peculiar fact that large numbers of homoplastic parathyroid transplantations in man are successful, i.e., in the sense that they alleviate or permanently cure tetany. In our opinion, all such cases or alleged successful homoplastic parathyroid transplantations are probably really unsuccessful in the sense that it is highly improbable that the grafted glands 'took' permanently. It seems more reasonable to presume that the implanted tissue tided the individual over a brief critical period until accessory parathyroids could function properly. Anatomical evidence for the existence of accessory parathyroids in man is meager. But judging by the numerous accounts in the literature of so-called successful homoplastic grafts (histological examination of such grafts later is rarely, if ever, made); it seems probable that such glands are present in many individuals. At any rate, our data from crisscross operations on cats and the evidence from the literature on the cure of human tetany by grafting indicate that wherever possible homoplastic grafting should be resorted to in cases of tetany in man. The procedure can do no harm and may do a very great deal of good. Table 5 summarizes the chief data obtained from crisscross operations.

DISCUSSION

The large per cent of cats which possess accessory parathyroids greatly complicates the correct interpretation of results obtained from transplantation where these animals are used for experimental material. Accessory glands are very difficult to find at autopsy, especially if there be much fat or thymus tissue present, and the smaller glands are practically impossible to find except by the method of serial section of the entire neck region which of course is not practicable in such an animal as the cat. We attribute all of our cases of apparent success in homoplastic transplantation of the parathyroids, i.e., success in the sense that the animal permanently recovered from the tetany symptoms following gland implantation, to the presence of minute masses

of accessory parathyroid which were present in these animals and became sufficiently functional to preserve life following gland removal. The homoplastically grafted glands, we believe, simply warded off fatal tetany until the accessories had time to properly function. This point of view is borne out by both the experimental and anatomical evidence. In several cases of recovery after parathyroid extirpation (and gland implantation) accessory parathyroids were found at autopsy—for instance, Cats 3 and 5 (table 2) and Cats 57, 60, and 75 (table 5). It is true that we were unable to find them in every such case, but nevertheless this does not alter our belief that they were present. Any one who has ever attempted to find accessories in cats at autopsy will appreciate the difficulties attending such a search. We have found accessory parathyroid glands in sections of the cervical thymus consisting of clusters of twenty or thirty parathyroid cells in cross-section, glands which would be practically invisible by macroscopic examination.

Besides the matter of accessory glands, there are other obscure and at present unknown factors which appear to play an important rôle in determining whether or not a given set of animals will or will not be favorable material for transplantation work. For illustration, a group of twelve cats was thyro-parathyroidectomized and transplanted (crisscross operation) within a week, and of this number two died, whereas ten survived. Two months afterward, a group of five animals was treated in a similar fashion within two days, and every animal died of tetany. The diet of both sets of animals was the same for four days before operation. The set of animals which died of tetany were obtained from a dealer in Philadelphia, and we do not know what their food was previous to the four days they were kept in the laboratory before operation. In one case the per cent of survivals was abnormally high, in the other case the per cent of fatalities was abnormally high. We present this data for what it may be worth, as we have no explanation which we believe adequate to cover the facts. It

may be added that conjunctivitis and colds were rife among this group of cats showing the high percentage of fatalities.

With regard to the few cases in which portions of the homoplastically transplanted parathyroids retained a fairly normal histological appearance despite several weeks in a foreign environment, we are in doubt as to whether or not they really were physiologically active. In only two cases, Cats 55 and 58 (table 5) were functional tests made. The transplanted tissue was removed after twenty-one days in one case and ten days in the other, but no untoward symptoms developed in either cat, thus indicating that accessory glands were probably present. In animal no. 75 (table 5) portions of both parathyroids persisted in fairly good condition. At autopsy of this animal, one small accessory parathyroid was found in the lower portion of the cervical thymus. It seems likely that had the graft been extirpated from this cat that the remaining accessory parathyroid would have been sufficient to keep the animal alive and in good condition.

In view of our other data on the fate of the graft in numerous other cases of homoplastic grafting of parathyroids, we are inclined to believe that only very rarely does a homoplastic graft of parathyroid tissue, or part of such a graft in cats at any rate, maintain any semblance to normal histological structure, and in those few cases in which such proved to be the case, it seems probable that if left unmolested for a longer time, the grafts would have eventually been resorbed.

SUMMARY AND CONCLUSION

1. Autoplastic parathyroid transplantation is successful in cats, both from the standpoint of tetany prevention and survival of the grafted tissue.

2. Six cats were thyro-parathyroidectomized and autoplastically grafted with the two external parathyroids. In two animals the glands were implanted into a pocket cut in the rectus abdominis muscle; in the remaining four the graft was made into the sternohyoid muscle. Four of the animals showed transient tetany, but recovered within a few days after grafting. Two cats showed no symptoms.

3. The grafts were reextirpated after thirty days. Two animals died of tetany, two showed no symptoms, one developed slight tetany shortly after graft removal, and in the remaining animal the graft could not be located.

4. Small accessory parathyroids were found in the thoracic thymus of two of the animals which failed to develop fatal tetany following reextirpation of the graft.

5. Histological examination showed that the grafts had 'taken' and were in excellent condition except for necrosis of the central area which had been replaced by connective tissue.

6. Twenty-eight cats were thyro-parathyroidectomized and homoplastically transplanted with two external parathyroids of other cats of the same sex. The grafts were of two types: those performed after the onset of tetany symptoms and those made before tetany developed.

7. Twelve animals were transplanted after the onset of tetany, six were grafted in the ear, and six, in the rectus abdominis muscle. All of the cats except two died of tetany. Of the two animals which recovered, one showed degeneration of the graft, but no tetany symptoms, owing probably to the presence of accessory glands; the graft persisted in fairly good condition in the remaining animal. It seems probable that the latter also possessed minute accessory parathyroids.

8. Sixteen cats were transplanted in the rectus abdominis muscle before the onset of tetany; eight survived, although most of the animals showed transient symptoms; the remaining eight died of tetany. The grafts of both fatal and non-fatal cases, except two, showed great degenerative changes.

9. Two cases which recovered showed portions of the transplanted parathyroids persisting in fair condition after a lapse of some weeks. One of the animals had a small accessory parathyroid present.

10. The experiments on crisscross operations which show 50 per cent of recovery from thyro-parathyroidectomy following transplantation (sixteen animals) indicate that in a

certain per cent of animals the grafts probably tide the animal over a critical period of a few days, enabling the accessory glands probably present (found to be present in several cases) to then become sufficiently active to preserve the life of the animal despite degeneration of the transplant later.

11. It is our belief, based upon our experimental findings in cats, that many of the reported cases of successful homoplastic parathyroid transplantation in man, i.e., successful, in the sense that the tetany was permanently cured, are due not to permanent survival of the graft, but to a purely temporary effect produced by absorption of the graft and its hormone which tided the individual over a critical period of a few days until the functionally deranged glands of the individual, or accessory parathyroids, could assert their activity.

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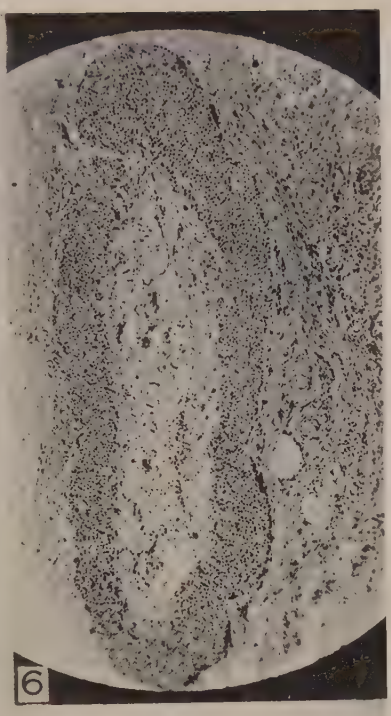
PLATE 1

EXPLANATION OF FIGURES

5 Photomicrograph of a transverse section of an autoplastic graft. × 75.
6 Transverse section of an autoplastic transplant. × 75.
7 Transverse section of a homoplastic graft of two parathyroids. × 75.

5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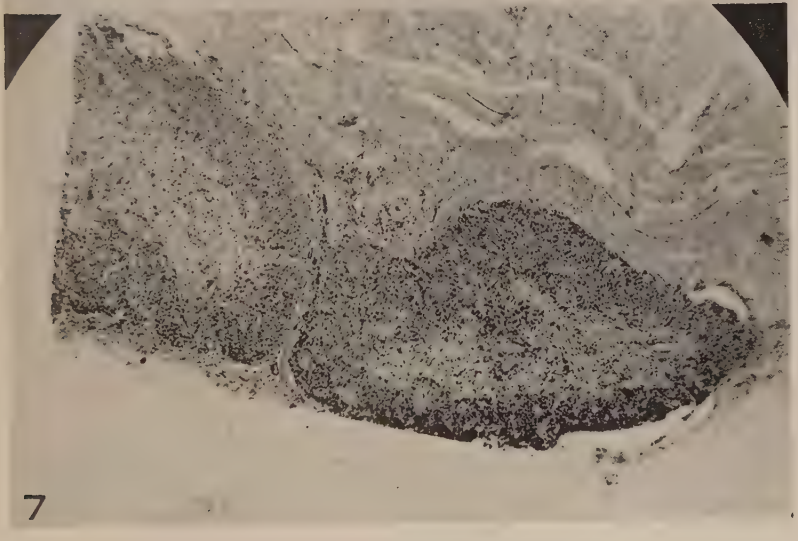
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Reprinted from the Proceedings of the Society for Experimental Biology and Medicine,
1925, xxiii, pp. 31-32

2815

A thyroid-adrenal interrelationship.

R. L. ZWEMER (Introduced by W. W. Swingle).

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Evidence has accumulated during the last few years that the cortical portion of the suprarenal complex of mammals is essential for life. This has been clearly demonstrated by the work of Houssay and Lewis,[1] Wislocki and Crowe,[2] and the present writer.[3] In a series of experiments performed upon forty cats, the writer was able to show clearly the great importance of cortical tissue for the maintenance of life. The following points were brought out: (1) Cats, from which the adrenals have been removed (in two stages 7 days apart), die within three days. (2) Unilaterally operated animals remain perfectly normal. (3) Total extirpation of one adrenal, and removal of the medullary portion of the other (after 7 days), leaving only the cortex, does not induce symptoms, the animals remain normal. (4) Removal of the remaining cortical tissue after an interval of 21 days to three months, causes death within three days. (5) Histological examination of the removed cortex showed no medullary tissue to be present in the majority of cases. (6) Transplantation of pieces of the cortex into animals with both adrenals removed, prolonged the life of the animals. During the course of the experiments mentioned, an interesting functional interrelationship between the suprarenal cortex and the thyroid gland was established. It was observed that thyroidectomized cats survived removal of the suprarenal glands much longer than animals with intact thyroid apparatus. In experiments on twenty animals this point was thoroughly investigated.

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[1] Houssay, B. H., and Lewis, J. T., *Am. J. Physiol.*, 1923, lxiv.

[2] Wislocki, G. B., and Crowe, S. J., Johns Hopkins Hosp. Bull., 1924, xxxv.

[3] Zwemer, R. L., *Anat. Record*, 1924, xxix.

The thyroid glands and the left adrenal were removed at one operation, ten days later the right adrenal was extirpated. Following thyroid removal the parathyroids were dissected free from their investing membranes in a medium of sterile Ringer's solution and blood serum[4] and transplanted into the neck muscles, to prevent tetany.

The life of animals from which the thyroids and adrenals had been removed was considerably prolonged over that of similarly operated cats in which the thyroids were left undisturbed. The average survival period of cats with the adrenals removed (in two stages 7 days apart) is three days, whereas the average survival of thyro-adrenalectomized cats is three times as long, or about nine days. One of these animals survived 22 days.

In order to determine the effects of feeding thyroid to normal animals, four adult cats were each fed four grains of dessicated thyroid (Parke, Davis & Co.) per day. Two animals received the thyroid for two weeks, two for one week. All showed an increased appetite and a decrease in weight, but no unfavorable symptoms of any kind were observed. The next step was to test the effect of feeding thyroid to adrenalectomized animals. The thyroid glands and left adrenal were removed, and, during the ten day interval between the removal of the left and right adrenals, the animals received four grains of dessicated thyroid per day. At the end of ten days the remaining adrenal was removed. Death resulted within 12 to 23 hours. This short survival period cannot be attributed to faulty technique, because in all cases the cats recovered from the anesthetic and behaved normally for some hours after the operation. Autopsies showed the wound areas to be clean, and the gross appearance of the viscera to be normal.

One animal developed typical symptoms of adrenal insufficiency and died 9 days after the first operation, although still retaining one adrenal gland. This case was complicated, however, by the occurrence of transient tetany on the third and fourth day after thyroid removal.

These experiments demonstrate a functional interrelation between the thyroid and adrenal glands, in that the life of animals deprived of their adrenals, can be greatly prolonged or shortened by absence or excess of the thyroid hormone.

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[4] Swingle, W. W., and Nicholas, J. S., *Am. J. Anat.*, 1925, xxxvi.

On the Origin of the Connective-tissue Ground-substance in the Chick Embryo.

By

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With Plates 44–7.

Szily (**26**) demonstrated in the early development of the chick embryo, as well as in certain other species, a cell-free, fibrous, connective-tissue ground-substance which was quite generally present, filling the various cavities of the embryonic body. This investigator was of the opinion that this material was intracellular in its origin, arising, as he believed, by a transformation of cell processes from any of the surrounding cells, without regard to the particular germ-layer to which they belonged, but previous to the advent of the mesenchyme cells.

In a previous communication (**5**) I have presented results which demonstrate, I believe, that in the Amphibia the process of connective-tissue formation is a purely intercellular one in which, first, a homogeneous intercellular ground-substance is formed, apparently as a result of the secretory activities of the surrounding embryonic cells, and, second, this secreted material is gradually transformed into the various types of connective tissue.

In view of the earlier work of Szily, as noted above, I felt it necessary to re-investigate the development of connective tissue in the chick, in order to see if, in my opinion, the process as there shown presented evidence contrary to that obtained from the study of the amphibian tissues. It may be stated

at this time that the results obtained in the present investigation are in harmony with the intercellular theory of connective-tissue development, and therefore in agreement with my previous work on the development of connective tissue in the Amphibia (**5**).

Material and Methods.

Access has been had to a large collection of serial sections of chick embryos ranging from 16–90 hours incubation. This material was preserved in sublimate-acetic (saturated aqueous solution of mercuric chloride with 5 per cent. glacial acetic), sectioned in paraffin either at 8μ or at 10μ and stained with Delafield's haematoxylin and orange G. For comparison a considerable number of embryos were preserved in Zenker's fluid (with 5 per cent. glacial acetic) and stained with the Mallory connective-tissue stain. In general it has been found that the sublimate-acetic fluid followed by the Delafield stain gives, on the whole, the most satisfactory results for chick tissues.

In addition to a study of the prepared material, considerable emphasis has been placed upon results obtained from the dissection of living chick embryos. The method employed in this phase of the investigation is briefly given below (p. 579) in connexion with the description of results obtained.

Study of the Prepared Material.

In fig. 1, Pl. 44, is shown a portion of a transverse section through an 11-somite embryo incubated 29 hours. The section is taken in the region of the 9th somite. It will be noted from the figure that at this stage a considerable area of the intercellular ground-substance is present surrounding the notochord and, in general, filling the spaces below the somites and lateral to the medullary cord. The surrounding cells are quite regular, rounded bodies which are devoid of projecting cell processes. There can be no question in such cases but that the ground-substance has arisen as an intercellular secretion

of certain surrounding cells and not from any direct transformation of cytoplasm. In this embryo a considerable degree of fibrillation is present in the ground-substance. A study of material obtained from a number of embryos shows that the degree of fibrillation varies considerably, although there is a very definite increase to be noted in the older embryos. Thus in some cases the ground-substance is apparently homogeneous, while in other embryos of the same age considerable fibrillation is present. I am of the opinion that this condition is due, at least in part, to the varying action of the preserving fluid. However, the point to be emphasized, which is very evident in this figure, is that the fibrillation unquestionably arises by changes which occur in the ground-substance.

A somewhat later stage is shown in fig. 2, Pl. 44, which is drawn from a transverse section of a 14-somite embryo at about the 36th hour of incubation. The section is also taken through the region of the 9th somite. Compared with the condition shown in fig. 1, Pl. 44, the ground-substance enclosing the notochord and filling the various spaces is more dense, and the fibrillation is also considerably more advanced. The distinction between the areas around the notochord filled with the fibrillated ground-substance and the empty cavities of the neural tube and the aorta is very sharp. It will be noted in a few cases that certain of the mesodermal cells lying in the peripheral layer of the somites have lost their spherical shape and assumed a more or less spindle shape with definite processes extending out into the ground-substance. Sections of this embryo taken anterior to the one here figured show, in general, more of the peripheral cells which are beginning a definite migration into the ground-substance. On the other hand, in sections of this embryo taken posterior to the one shown the transformation of the peripheral cells is less advanced than shown in this figure.

In fig. 3, Pl. 45, we have a portion of a transverse section through the tail region of an 18-somite embryo, incubated about 42 hours. The section shown in this figure is taken a considerable distance to the right of the median line as seen

under the microscope, and exhibits the separation of the splanchnic and somatic layers of the mesoderm to form the extra-embryonic coelom on that side of the embryo. In this figure it is desired to call particular attention to the dense layer of heavily fibrillated ground-substance lying between the mesoderm and ectoderm, and between the mesoderm and endoderm, thus uniting all the layers and forming a compact whole. As in the previous figure the contrast between the empty coelomic cavity and the completely filled areas between the mesoderm and the other two layers is very marked. The migration of the mesoderm cells into the ground-substance has not begun as yet in the region figured.

In fig. 4, Pl. 45, is shown a portion of a frontal section through a 27-somite embryo of 48 hours incubation. This section is taken at the level of the notochord, and gives a very clear picture of the relations between the ground-substance, notochord, and mesodermal somites. The figure gives added confirmation to that noted previously in the transverse sections, namely the presence of the large area of ground-substance surrounding the notochord and extending around the somites. In this region, at the stage shown, this material is cell-free, but it can be seen that long processes from several of the mesoderm cells are beginning to extend considerable distances into it. Some of these cell processes in the ground-substance are drawn out quite fine, but it is possible to distinguish between the cytoplasmic process and the fibres present in the ground-substance. There can be no doubt that the two are independent structures.

Fig. 5, Pl. 46, is a portion of a transverse section through the region of the 21st somite of a 27-somite embryo, incubated 48 hours. The portion figured lies ventral to the neural tube and shows a section of the notochord. This region is of particular interest at this stage because some of the peripheral mesoderm cells from the pair of somites are migrating into the cell-free area of the ground-substance. A comparison of this figure with figs. 1 and 2 shows the changes that are taking place. It is evident that these cells in their movements from

the cell groups, just as was previously shown in the case of the frog embryo (**5**), make use of the ground-substance as a supporting substratum. Their shape has undergone considerable modification from that of the earlier condition, and long processes have formed which extend into the ground-substance for considerable distances. Emphasis should be laid upon the fact that the cytoplasm of the migrating mesoderm cells, even when drawn out to a very fine process, can be differentiated from the surrounding ground-substance, so that it is possible to be certain of the independence of the two materials.

A condition such as is shown with particular clearness in fig. 5, Pl. 46, gives striking evidence that the movements of these embryonic cells in the chick embryo are governed by the same factors as are the living cells in tissue cultures. In the latter condition it has been conclusively demonstrated by L. Loeb, Harrison, and others that cells are positively stereotropic, and in order for their migration to take place away from the cell groups it is necessary that a supporting material of some sort be provided.

If, for example, the cells in a tissue culture are suspended in a liquid medium so that they are not in contact with any supporting material, they will show no tendency to leave the cell groups and move out into the liquid. On the other hand, if the cells are surrounded by a more or less solid medium such as a blood or plasma clot, or if in a liquid medium a fibrillar material, such as a filament from a spider web, is provided the cells in contact with the supporting material will, in general, begin to migrate away from the mass of tissue and out into the surrounding areas. Furthermore, in such a movement, when the cells make use of the fibrillar materials, it is clear that their shape represents an adjustment to the environment. Thus when they are quiescent in the group they remain as spherical bodies, but when they are undergoing active movement they become spindle shaped, with the long axis parallel to the direction they are moving. This fact is shown to particular advantage in cases where the cells are moving along a single fibre, such as in a spider web (**13**).

The study of connective-tissue development, both in the frog and the chick, goes to show clearly that the behaviour of the embryonic cells in the developing animal with regard to migration is the same as in the tissue cultures. It is necessary that the cells in the embryo have a supporting material in order to enable them to leave the cell groups and migrate into and through other regions of the embryo. Such a material is provided in the embryo from the earliest stages by the ground-substance which arises as a cell secretion; structureless at first, but variously modified later as the cells wander through it.

In fig. 6, Pl. 46, is shown a portion of a transverse section through an embryo of 36 somites at the 72nd hour of incubation. The section is through the region of the 21st somite. At the top of the figure a portion of the neural tube is shown. Lying below the latter a transverse section of the notochord may be noted. The latter is surrounded by the fibrillar intercellular material which has arisen by a modification of the secreted ground-substance and which is now infiltrated with great numbers of mesenchyme cells. A comparison of this figure with those previously described, particularly fig. 5, Pl. 46, demonstrates very clearly the marked changes which have taken place in this region of the embryo, due to the active median migration of the mesenchyme cells from the region of the somite on each side.

Mall in his study of the development of connective tissue in the tadpole and pig (**20**) shows a stage similar to fig. 6, Pl. 46, but interprets it differently. He holds, in accordance with the exoplasmic theory of connective-tissue formation, that the fibrillar material, such as is shown in fig. 6, Pl. 46, arises from a syncytium of the mesenchyme cells and therefore that it is really a modified exoplasm of the cells, intercellular in position, but since it arises, as he believes, by a transformation of the peripheral cytoplasm, it is really intracellular in its formation. When the fact is taken into account that the forerunner of the cell-containing, fibrillar material shown in fig. 6, Pl. 46, is the secreted, cell-free ground-substance which may be found generally present throughout the embryo, it is

clear that the formation of this material must be the result of an intercellular action.

It is interesting to note in connexion with the so-called mesenchyme syncytia postulated by the exoplasmic theory, certain studies of W. H. Lewis, who, working with mesenchyme tissue of chick embryos in tissue cultures (**18**), shows in a number of cases that cells, which to all appearances were completely fused to form a definite syncytium, were really separate entities which retained their individuality at all times. What appeared to be a syncytium was in reality only a group of cells in close apposition to each other. In a later paper (**19**) Lewis says he is convinced that the mesenchyme cells of chicks do not form syncytia in the tissue cultures, and he thinks it highly probable that the same thing is true with regard both to the embryos of birds and of mammals. Whether or not it is true that the mesenchyme cells never form syncytia, it can be demonstrated that a basic, more or less fibrillated ground-substance is present in the embryo previous to any mesenchymal syncytium. The latter, therefore, if such occurs, is not responsible for the formation of the ground-substance.

In considering the discrepancy between the results of Szily (**26**) and those here recorded, the chief difference appears to be due to the failure of Szily to recognize the earliest stages in the formation of the ground-substance. According to the present findings, the ' zellfreie faserige Stützgewebe ' of Szily is not the primary stage, but previous to this condition there is present in the chick embryo a homogeneous ground-substance which gradually becomes fibrillated. The fibres which form are purely intercellular, having no direct connexion with cells but arising by a direct transformation of the ground-substance.

Condition of the Ground-substance in the Heart.—The early developmental stages of the chick heart constitute probably the most satisfactory region of the embryo for demonstrating the presence of a large amount of the cell-free ground-substance. Szily (**26**) clearly showed the presence of this material in the heart. Also Davis at the 1924 meeting of the American Association of Anatomists (**8**) presented

results showing that a homogeneous transparent material, which he terms ' cardiac jelly ', is present in the living heart of 48- to 72-hour chick embryos. He sectioned the living heart in Locke's solution and found that a fine probe ' insinuated between the myocardium and endocardium meets with resistance, and separation of the two layers is accomplished with great difficulty '. He reported, however, that he was unable to demonstrate this material microscopically either in fresh preparations or in material which had been preserved and sectioned. He concludes ' that the substance between the myocardium and endocardium is a homogeneous transparent jelly '.

I have found no difficulty in confirming these observations of Davis on the presence of this material in the living hearts, and also in demonstrating it microscopically as shown previously by Szily. In figs. 7, 8, and 9, Pl. 47, are shown three sections through portions of the developing heart as different stages. Fig. 7, Pl. 47, shows an early stage taken from a 27-hour embryo. In this figure a cell-free layer of ground-substance of considerable thickness is to be noted lying between the endocardium and myocardium. In part, this material even under the magnification appears homogeneous, in part it is fibrillar. Since at this stage the cells of both the myocardium and endocardium are definite rounded bodies, it is possible to say with certainty that they are not in direct connexion with the fibres. It is clear, therefore, that the fibrillation arises by a change in the character of the ground-substance. The relative amount of fibrillation varies somewhat in different preparations, and, as has been previously noted, this may be due in some degree at least to the action of the killing fluid.

In fig. 8, Pl. 47, a section is shown through a portion of the heart of a 42-hour embryo. In comparison with the earlier stage, sections through the heart of an embryo of this stage consistently show, as evidenced in the figure, a marked increase in the density and in the fibrillation of the ground-substance. Here, again, it is clear, as in the earlier stage, that the development of the fibres is due to a change in the character of the inter-

cellular substance, and not to the transformation of cell processes or any other direct cell connexion.

In fig. 9, Pl. 47, a section is shown through a portion of the heart of a 72-hour embryo. Somewhat previous to this mesenchyme cells have begun a migration into the intercellular ground-substance. To the left of the figure a considerable number of these more or less spindle-shaped cells are shown lying in the ground-substance and apparently stretching out along the fibres. To the right of the figure a portion of the ground-substance is shown in which considerable areas are still cell-free.

Study of the Intercellular Ground-substance in Living Chick Embryos.

It is possible to demonstrate the presence of a transparent, gelatinous material in living chick embryos from very early stages just as it is in frog embryos (**4**). The method employed has been as follows. The egg is opened in a dish of warm normal saline. Then the blastodermic area with the embryo is cut from the yolk and floated into a Syracuse dish with some of the same fluid. In some cases dishes have been used which contained a layer of paraffin to which the blastoderm could be pinned in order to keep it in position. In other cases the blastoderm has been held in position by means of lead weights. The examination of the embryos had been done under a Zeiss binocular microscope using either the intense direct illumination of a small arc lamp, or the indirect illumination from the mirror. Dissection of the embryo has been accomplished by the use of glass needles drawn to the desired fineness over a small gas flame according to the methods used by Chambers (**7**) for microdissection needles. Very fine scissors have also been used. By these methods it has been found possible to carry out almost any dissection desired on the various stages.

The earliest stage dissected has been that of the primitive streak, ranging from 15–18 hours incubation. The dissection of such an embryo permits the demonstration of an inter-

cellular ground-substance in various ways. Thus it will be found by careful manipulation with fine needles that the ectoderm in the embryonic area is adherent to an underlying transparent material between the ectoderm and mesoderm so that an attempt to remove the former meets with resistance. It is apparent from a study of both living and prepared material that the mesoderm cells are separated from the ectoderm except possibly in the immediate vicinity of the primitive streak. If transverse sections of the embryo through the primitive streak are made, and the sections manipulated with needles it will be found that the ectoderm, mesoderm, and endoderm tend to keep their relative positions, and any attempt to compress or extend meets with resistance. Furthermore, when the pressure or tension is released the germ-layers tend at once to assume the original position. In separating groups of the embryonic cells, it will be found that they are adherent to each other. This is apparently due not to an adhesion of the limiting cell membranes but to the presence of a transparent intercellular material, small areas of which under favourable conditions can be detected in various regions between the cells.

An embryo of 48-hours incubation is one of the best stages for demonstration of the ground-substance in the living embryo, just as it is in the prepared material. An embryo at this age is large enough to permit a comparatively easy dissection. Transverse sections at various levels afford clear demonstrations of the presence of a homogeneous ground-substance filling the spaces between the germ-layers and in various other cavities of the embryonic body. The results from such an examination confirm those obtained from the study of the prepared material. Thus if one examines the cut edge of a transverse section of a living embryo taken posterior to the cervical flexure at about the 15th somite, as shown in fig. 10, Pl. 47, it is possible by means of fine glass needles to demonstrate the presence of the jelly in the area surrounding the notochord, and also in the space between ectoderm and mesoderm. Any attempt to alter the position of the notochord meets with resistance, as does also an attempt to strip away the

ectoderm. On the other hand, the needles can easily be inserted into the extra-embryonic coelomic cavities between the somatic and splanchnic layers of the mesoderm or into the neural canal, all of which are apparently entirely free from any enclosed material. Furthermore, just as in the earlier stages any attempt to alter the position of the germ-layers or other structures in an embryo of this stage meets with resistance, so one must conclude from such an examination that the intercellular ground-substance is of quite general occurrence throughout the embryonic body.

Discussion.

The above results obtained from the study of both the prepared and living material present a very clear picture as to the origin of the connective tissues in the chick. The primary fact is the presence of an intercellular ground-substance throughout the embryo from the earliest stages of development, which arises as a secretion from the cells of the various germ-layers. In some cases it appears that the mesoderm cells are not concerned in the formation of the ground-substance, for areas of the material may be found which are situated a considerable distance from the cells of this layer. On the other hand, in the development of the heart, it is apparent that the abundant formation of the ground-substance must be due to the mesoderm cells.

The ground-substance in the early stages appears almost structureless save for a slight fibrillation. Because of this fact it is not easy of detection in the prepared material even under favourable conditions of staining, but it can be demonstrated by dissection of living embryos. The degree of fibrillation, although subject to some variation in embryos of the same stage, shows a definite increase in the older embryos, and it can be demonstrated that this phenomenon is due to changes which take place in the ground-substance and therefore independent of direct cellular connexion. There has been no question for some time but that in the later stages the development of

connective tissue took place by an intercellular action. Since it is also apparent that the same condition is true for the earlier stages as well, it must be concluded that connective-tissue formation is purely an intercellular process.

In connexion with the connective-tissue problem attention should be called to a noteworthy contribution by Harrison (**14**) obtained from his recent study of the development of the balancer in Amblystoma, which, as he shows, affords ideal material for a study of connective-tissue development. The results presented afford a conclusive demonstration of the presence of an intercellular, secreted ground-substance and the gradual transformation, by changes in the material itself, of a peripheral region to form a basement membrane. In the balancer it is clear that the undifferentiated ground-substance arises as an intercellular formation of the mesoderm cells. The peripheral basement membrane which arises from this material is ' composed of a dense felt of reticulum fibres ' and is formed under the influence of the surrounding epithelial cells. As to the type of influence exerted by the epithelial cells upon the ground-substance in order to form the basement membrane, Harrison ' naturally thinks of an enzyme action which condenses or coagulates the diffuse intercellular ground-substance transforming it into a fibrillar tissue which gives almost the same chemical reactions as reticulum '.

' The balancer membrane thus affords a clear proof that connective-tissue fibres take origin in an amorphous ground-substance, independently of any direct action on the part of the mesenchyme cells. The fibres composing the membrane are neither intracellular nor are they formed in any particular outside layer which could be called exoplasmic, unless the whole of the intercellular ground-substance be designated as such.' It is difficult to see how a more conclusive result could be obtained, or one which was more in harmony with the position I have maintained for several years.

I am of the opinion that considering the results which are now available the question of the origin of connective tissue must be regarded as settled in favour of the intercellular

theory. The next step is the definite linking up by conclusive chemical studies of the process of connective-tissue formation in the embryo with the process of wound healing as well as various other pathological conditions in the mature animal in which, in some cases at least, the transformed plasma clot plays such an important role. In this connexion it may be worth while to quote a paragraph from a previous paper (**5**).

'From the morphological standpoint the results of the present study indicate that the formation of connective tissue in the amphibian embryo is similar to the process which takes place in transformation of the plasma clot. The intercellular ground-substance of developing connective tissue may therefore be compared in its morphology to the plasma clot. This ground-substance when first formed appears homogeneous or with a fine fibrillation. The process of transformation into a fibrous tissue is a progressive one. The fibrillation increases, bundles of fibres are formed, and in time the entire ground-substance, which at first showed such a high degree of homogeneity, becomes transformed into a fibrous tissue. It is indicated that this transformation occurs as the results of the introduction of mechanical factors in the embryo. These factors may be due to certain lines of tension in the embryo corresponding to the inherent polarity of the organism or, just as in the plasma clot, the movements of the cells through the ground-substance may introduce mechanical factors which aid in the transformation of the ground-substance into a fibrous tissue. The cells, however, are to be regarded primarily as assimilative and secretory agents, chiefly concerned in the formation of the undifferentiated ground-substance.'

The fact that the primary factor in connective-tissue formation is a secreted intercellular ground-substance, which is secondarily invaded by mesenchyme cells and modified in various ways, necessarily leads to an application of this knowledge to certain features of development other than connective-tissue formation. In the first place, taking into account the fact (p. 575) that cells require a supporting material when moving away from cell groups, it is apparent that the presence

of the secreted, gelatinous ground-substance in an embryo from the earliest stages is the sine qua non for the migration of isolated embryonic cells away from the cell groups and the development of mesenchyme throughout the body. For example, as is well known in the chick embryo, the mesoderm is first differentiated along the primitive streak. From this region the cells migrate laterally and anteriorly between the ectoderm and endoderm. Now from the dissection of the living embryo it can be demonstrated that the space between the ectoderm and endoderm even at this very early stage contains a gelatinous supporting material, the primitive ground-substance. The conclusion appears warranted that a type of cellular movement, such as is exhibited by the mesoderm cells at this stage in which isolated cells move away from the cell groups, is dependent in the embryo, just as it is in tissue cultures, upon the presence of the secreted ground-substance.

Another striking example in the embryo of the relations between the ground-substance and cell movement may be noted in the outgrowth of the nerve-fibre from the neuroblast as demonstrated by Harrison in tissue cultures (**12**). In this type of movement the cell-body retains its position in the wall of the neural tube, but gradually form a process which extends a considerable distance from the cell-body as a nerve-fibre.

Harrison in a later paper (**13**) says: 'With regard to the movements of the growing nerve-fibre the evidence . . . is not quite so varied, but it is sufficient to warrant the conclusion that also this protoplasm is stereotropic. No free outgrowth of nerves in a fluid medium has ever been observed, while such solids as the fibrin clot and smooth glass surfaces serve readily to support them, as do the surfaces of the larger cell-masses and the interstitial protoplasmic network inside the embryo.'

In connexion with the normal growth of the nerve-fibre in the embryo an examination of the figures of Held as given in his monograph on the development of nerves in the vertebrates (**16**) is very instructive in that they show many instances

in which the growing nerve-fibres are apparently utilizing a more or less fibrillated ground-substance in their extension through the embryonic spaces.

Finally, it should be noted that the early and abundant secretion of a ground-substance by the embryonic cells may be an important factor in the growth of the embryo. In other words, it is possible that, at certain stages of development, the growth of an embryo may not be due so much to an increase in the number of cells formed by cell division as to an increase in the amount of the secreted ground-substance. If, for example, one compares a chick embryo in the primitive streak-stage with a 48-hour embryo, a striking feature is the comparatively great increase in the amount of the ground-substance present. On this basis one may postulate in development of the chick a series of cycles in which a period of cell division in certain regions of an embryo will be followed in turn by a period of inactivity as regards cell division, but a period in which the assimilative and secretory processes of the cells attain their maximum activity thus resulting in an abundant formation of the ground-substance. The latter is at once utilized as a supporting material and as the basic substance, under the influence of the invading cells, for the formation of connective tissue and various other structures.

Summary.

1. In the chick, just as has been previously shown in the amphibian embryo, the forerunner of the connective tissues is a transparent, gelatinous, cell-free ground-substance which, in general, pervades the embryonic body from very early stages of development.

2. This ground-substance can be demonstrated in various regions of the body previous to the appearance of the mesenchyme cells. It is evidently formed as a secretion of the cells of the various germ-layers. The data at hand indicate no possibility that it arises either from a syncytium or by a direct transformation of the cytoplasm.

3. In the early stages the ground-substance appears, for the most part, homogeneous, but with some fibrillation. In the later stages a progressive increase in the fibrillation is noted. It is possible to show that the development of fibres in the ground-substance is due to changes in the ground-substance itself, and not to a direct transformation of cytoplasm.

4. The formation of the ground-substance is followed by the invasion of the mesenchyme cells which, using it as a supporting material, apparently in the same way that cells utilize the plasma clot in tissue cultures, move through and modify it in various ways.

5. Emphasis is laid upon the fact that the presence of the ground-substance through the embryo, together with the known positive stereotropism of cells as exhibited in tissue cultures, throws light upon certain features of early development, particularly cell movements and increase in body size.

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DESCRIPTION OF PLATES 44, 45, 46, AND 47.

Abbreviations (which apply to all figures).

AOR., aorta; ECT., ectoderm; END., endoderm; M.C., mesoderm cell MES., mesoderm; NC., notochord; N.T., neural tube; G.S., ground-substance; E.E.C., extra-embryonic coelom; EN., endocardium; MYO., myocardium.

Plate 44.

Fig. 1.—Portion of a transverse section of a 29-hour, 11-somite chick embryo in the region of the 9th somite. ×634. The notochord is shown surrounded by a more or less fibrillated, cell-free area of the ground-substance which extends laterally on each side under the somites and dorsally between the neural tube and the somite.

Fig. 2.—Portion of a transverse section of a 36-hour, 14-somite chick embryo in the region of the 9th somite. ×317. As compared with fig. 1 the ground-substance shows a greater density and fibrillation. It is practically cell-free, but certain cells in the peripheral layer of the somites can be seen to be assuming a spindle-shape and to extend out into the ground-substance.

Plate 45.

Fig. 3.—Portion of a transverse section of a 42-hour, 18-somite chick embryo in the tail region. ×317. The figure is taken a considerable distance to one side of the mid-line and shows a portion of the extra-embryonic coelomic cavity of that side. Between the ectoderm and the mesoderm and between the mesoderm and the endoderm the space is filled with a dense fibrillated ground-substance which stands out in marked contrast to the empty space of the coelomic cavity.

Fig. 4.—Portion of a frontal section of a 48-hour, 27-somite chick embryo at the level of the notochord. ×317. The notochord is shown embedded in considerable area of dense fibrillated cell-free ground-substance into which a considerable number of the mesodermal cells from the somites are beginning to extend long processes. It is possible to differentiate between the fibres in the ground-substance and the cell processes and to be sure that the two structures are independent.

Plate 46.

Fig. 5.—Portion of a transverse section of a 48-hour, 27-somite chick embryo in the region of the 21st somite. ×634. In this figure the beginning of the migration of the mesodermal cells into the area of the ground-substance surrounding the notochord is to be seen.

Fig. 6.—Portion of the transverse section of a 72-hour, 36-somite chick embryo in the region of the 21st somite. ×634. This region is the same as in fig. 5, and shows a considerably later stage in the migration of the mesodermal cells into the ground-substance surrounding the notochord.

Plate 47.

Fig. 7.—Transverse section through the developing heart in a 27-hour chick embryo. ×317. A considerable area of more or less fibrillated ground-substance is shown lying between the endocardium and myocardium.

Fig. 8.—Transverse section through the heart of a 42-hour chick embryo. ×317. The figure shows a large area of dense and fibrillated cell-free ground-substance lying between the endocardium and myocardium.

Fig. 9.—Transverse section through the heart of a 72-hour chick embryo. ×634. To the left of the figure is shown the invasion of the fibrillated ground-substance by the mesoderm cells, and to the right a considerable area of the cell-free ground-substance.

Fig. 10.—A transverse section through a living 48-hour chick embryo in the region of the 15th somite, just posterior to the cervical flexure. ×15±. In such a section the presence of the gelatinous ground-substance can be detected in the area surrounding the notochord and lying between the ectoderm and mesoderm and between the mesoderm and endoderm. These areas filled with the ground-substance are clearly differentiated from empty cavities such as those of the extra-embryonic coelom and of the neural tube.

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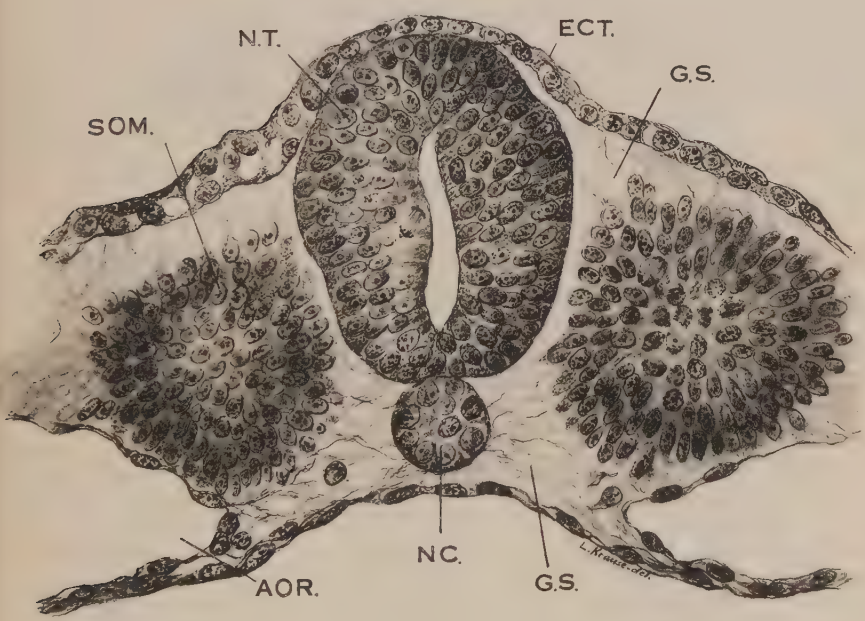
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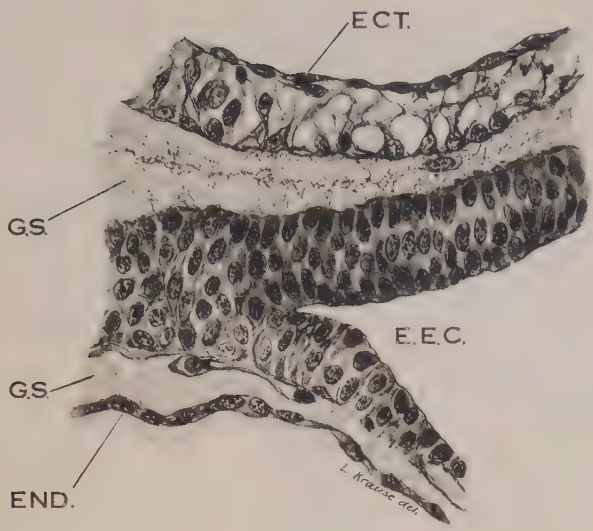

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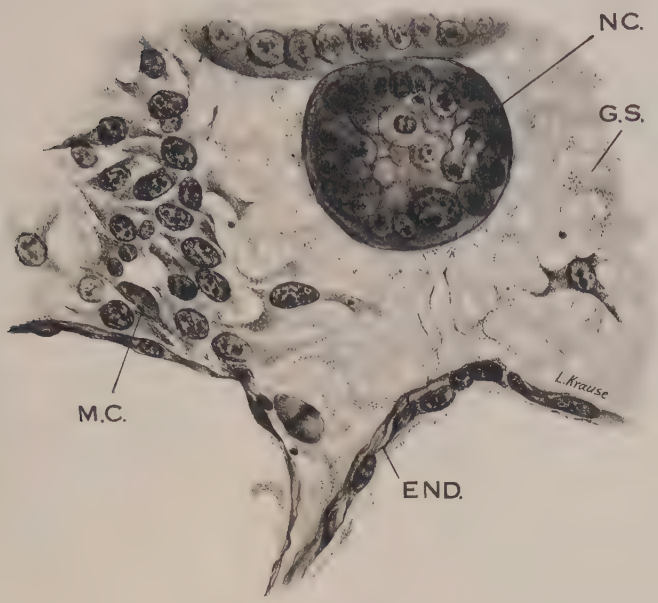

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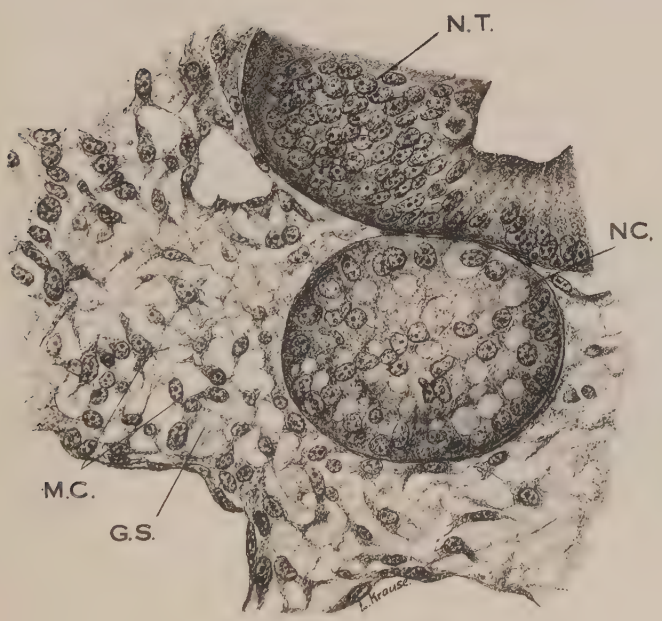

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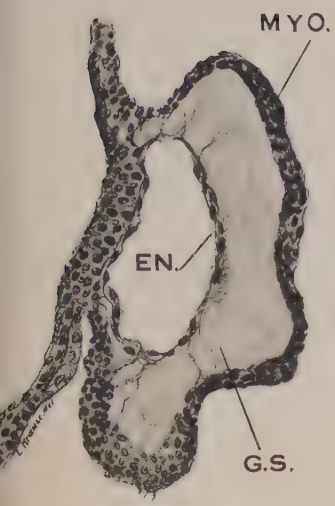
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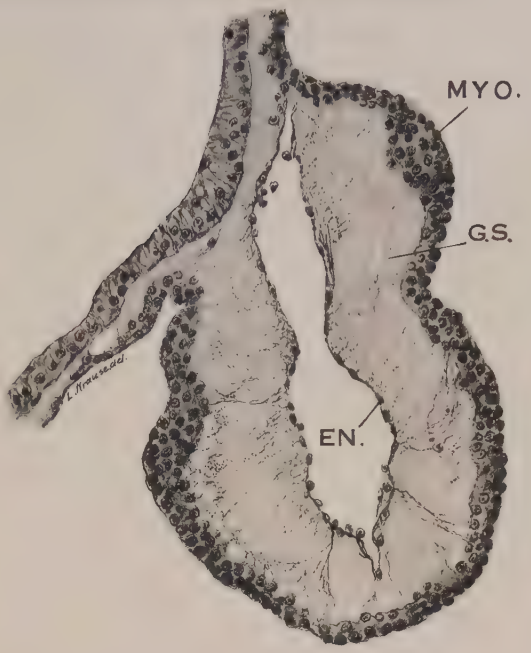
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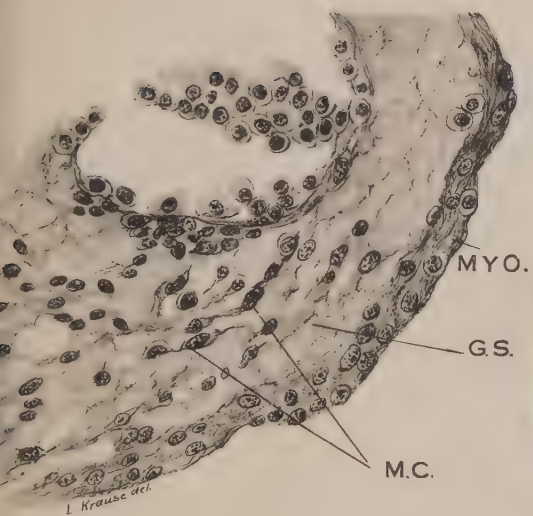
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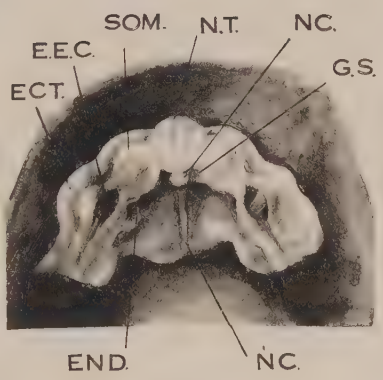
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Reprinted from the Proceedings of the Society for Experimental Biology and Medicine,
1925, xxiii, pp. 96-97

2844

A new culture medium for tissues grown in vitro.

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Harrison[1] demonstrated the growth of embryonic amphibian nerve tissue in the clotted lymph obtained from an adult frog. Burrows[2] demonstrated the growth of embryonic chick tissue in blood plasma obtained from the adult hen. In most of the experiments with tissue cultures since that time the blood plasma of various animals has been utilized either alone or in combination with certain tissue extracts as a culture medium. Some investigators, notably W. H. and M. R. Lewis, have used saline solutions, such as the Locke-Lewis solution, as a culture medium.

We have recently found that the peritoneal exudate of certain animals affords a culture medium which, in the experiments thus far made, gives evidence of being superior to any medium heretofore used. In the first place, this exudate apparently affords a more natural environment for the living tissues, with a corresponding increase in cell activity and length of survival *in vitro*. Also, the method of obtaining the exudate is comparatively simple. In the case of the frog, a considerable amount (about 0.5 cc.) may usually be secured from an anesthetized animal by drawing up the fluid from near the posterior end of the peritoneal

* This work has been done in connection with a grant from the National Tuberculosis Association.

1 Harrison, R. G., *J. Exp. Zool.*, 1910, ix, 787.

2 Burrows, M. T., *J. Exp. Zool.*, 1911, x, 63.

cavity with a fine sterile pipette. In the case of the guinea pig, the animal is fastened to a board, and the exudate then secured by means of a capillary pipette inserted through the ventral surface of the body wall. The tip of the pipette should just barely reach into the peritoneal cavity. Suction is applied by the operator through a rubber tube attached to the pipette. The same animal may be used repeatedly for securing the exudate, and it apparently is very little disturbed by the operation. In order to secure a more abundant exudate various substances such as blood, saline solution, etc., may be injected into the peritoneal cavity a few hours before the operation.

Finally, the exudate is more easily handled after it has been secured than is blood plasma, inasmuch as elaborate precautions are not necessary to prevent its clotting. In cases of the abdominal wall puncture, where the operation is not a clean one, a blood vessel may be pierced, and as a result, varying quantities of blood will be mixed with the exudate. This causes a more rapid clotting than with the exudate alone. The appearance of the exudate varies according to the number of white blood cells present. These may be readily thrown down by centrifuging, and the clear liquid used, or the exudate may be used without centrifuging. The experiments to date show that the white cells in the medium do not prevent the activity of the tissues *in vitro*. Experiments are in progress which, it is hoped, will furnish additional evidence on the use of various exudates as culture media.

THE EFFECT OF REVERSING THE MEDIO-LATERAL OR TRANSVERSE AXIS OF THE FORE-LIMB BUD IN THE SALAMANDER EMBRYO (AMBLYSTOMA PUNCTATUM LINN.).

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With 39 figures in the text.

I. Introduction.

The publication of Braus's first papers on transplantation of limbs in 1903 and 1904 has stimulated either directly or indirectly a long line of investigations dealing with some of the most fundamental problems of organic form. One phase of the subject, which was only lightly touched upon by Braus, has to do with the properties underlying symmetry, and to this much attention has recently been directed. The present study is a further contribution to this general field.

Previous experiments[1]) with the embryo of the spotted salamander (*Amblystoma punctatum* Linn.) have shown that the disc of mesoderm, constituting the fore-limb bud before it begins to grow out has a definite axial polarization in an antero-posterior (cranio-caudal) direction, while the polarity of its dorso-ventral axis is either not yet determined or is easily reversible. This conclusion was based upon a large number of experiments in which limb buds were transplanted, with various orientations, in both normal and abnormal location. Under these circumstances the transplanted limbs were found to develop in accordance with certain definite rules[2]), which were interpreted to mean that the eventual asymmetry or laterality of the limb bud is determined by two factors (or forces) acting approximately at right angles to one another, one located in the transplant itself and the other in the tissue surrounding it[3]).

[1]) Harrison, '17 and '21.

[2]) Harrison, '21, p. 4.

[3]) We are concerned here with the immediate surroundings and not with the organism as a whole. The work of Nicholas (22) in changing the orientation of a ring of tissue surrounding the limb bud shows this very clearly. It is necessary to emphasize this here because the distinction has not always been borne in mind.

Since this work was published a number of related studies have appeared[1]), so that it is now possible to take a more comprehensive view of the subject.

Working at the same time independently and on an entirely different object, the anchor plates of the Synaptidae, WILHELMI ('20) came to a conclusion somewhat similar to the above. She postulates two determining factors, one of polarity and another of symmetry, which act at right angles to each other. Though acting independently, both are assumed to be located outside the plate itself in the anchor which is attached to it. In a later publication WILHELMI ('22) gives an interpretation of my limb experiments, based upon her own views, by calling into play a symmetry factor, which is supposed to act a distance, either from the limb of one side of the body to that of the other or from the normal limb to a heterotopically implanted limb, five or six body segments from it. Interesting as this comparison may be, there is at present no evidence for the existence of influences of this kind acting at such distances, and they seem far less probable than the direct action of the arroundings.

BRANDT ('23 and '24) working with *Triton taeniatus* has shown that the fore-limb bud has the polarity of both dorso-ventral and antero-posterior axes determined in the tail-bud stage. In this species there is thus a precocious axial determination, as compared with *Amblystoma punctatum*, and in order to obtain limb buds with an indifferent dorso-ventral axis, it is necessary to go back to the early neurula (medullary plate) stage[2]). At this period a right limb bud implanted on the left side with its dorso-ventral axis inverted develops into a left limb. It is not possible, however, to state from this experiment whether the antero-posterior axis is fixed or not, and in the absence of experiments with all four orientations it will not be safe to draw conclusions regarding the relative time of polarization of the two axes.

Our knowledge of the corresponding features in the development of the anuran limb is in the same state, for while GRÄPER ('22a) has experimented extensively with several different species, the number of positive cases in which the orientation of the graft is given is small — only eleven — and these include only heteropleural trans-

[1]) Cf. GRÄPER, WILHELMI, BRANDT, v. UBISCH, MILOJEVIĆ, WEISS, PRZIBRAM.

[2]) BRANDT (op. cit. p. 524) calls attention to the great difference between *T. taeniatus* and *Amblystoma* (Axolotl) with respect to the time of development of the anterior limb, and advances the view that this difference may be correlated with a difference in the time of the determination of the dorso-ventral axis, thus accounting for the difference in the behavior of the transplanted buds. Unfortunately, however, this explanation will not hold, since in *A. punctatum*, the form used in my experiments, development of the limb is precocious, like that of *Triton*, and takes place very much earlier than in the Axolotl.

plantations, i.e., those in which the graft was placed on the opposite side of the body. GRÄPER experimented with the hind-limb of *Rana* and *Bufo*, using limb buds that varied in length from half to twice the diameter of the base (0,5/1—2/1). Most of the limbs in these cases retained their original asymmetry *(Seitenqualität)*, showing that both axes are often already fixed at that stage *(Querschnittsdetermination)*, but in another series of six cases in which the size of the limb varied from 0,5/1 to 1,5/1, the asymmetry of the grafted buds became reversed, showing that in these cases at least one of the two axes had remained indifferent. The anuran hind-limb at these stages is therefore in a transitional period, and here again a much more comprehensive study will have to be made before we can say whether the polarity of one axis is determined before that of the other. There is, however, nothing in GRÄPER's results that is contrary to the conception of axial relations given in my previous papers[1]).

In experimenting with regenerating limb buds of *Triton cristatus*, MILOJEVIĆ ('24) has shown that the blastema from which the limb regenerates first passes through an indifferent stage without axial differentiation. Thus if an appendage be cut off before a certain critical point and reimplanted after being rotated 180° about its long axis, a normal limb will develop, whereas after this critical stage the effect of the rotation makes itself felt. According to MILOJEVIĆ both the radio-ulnar and the dorso-palmar axes are determined at the same time. It is interesting also to note that before the critical point, the blastema is not even determined as regards the kind of a limb that will develop out of it, for the growing tip of the regenerating fore-limb will enter into the formation of a normal hind-limb if planted on the stump of the latter. If grafted after the critical period the transplanted material develops in accordance with its origin.

None of the above cited experiments give any information regarding the third or medio-lateral axis of the limb bud which becomes the proximo-distal axis of the growing limb. Our knowledge of this question has been based almost wholly upon the study of regeneration[2]), especially upon the phenomenon of "*Bruchdreifachbildung*" which has been fully treated by PRZIBRAM and DELLA VALLE. At the time my own experiments with carefully oriented transplants were made it did not seem practicable to reverse this axis, for with the technique then

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[1]) The one case which GRÄPER cites (op. cit. p. 304) in which a graft implanted dorso-dorsally became reversed, may well be like certain cases of my own in which this result occurred through the formation of a reduplicating bud of opposite asymmetry followed by resorption of the primary bud at an early stage (HARRISON, '21, p. 49).

[2]) Cf. experiments by MORGAN, LISSITZKY, and KURZ.

employed this would have necessitated placing the ectoderm inside the mesoderm. Although it was found that mesoderm from the limb region when inoculated alone in a pocket of skin on the flank of the embryo would give rise to a limb, in the earlier experiments of this kind, made with embryos in stage 29—31, it was not feasible to orient the engrafted material. In the later experiments, which are here reported, this difficulty was overcome.

Meanwhile GRÄPER ('22b) has subjected the condition of the growing axis of the anuran limb to study, using for the purpose mostly hind-limbs which were just beginning to show the broadening and flattening of the foot. The limb was removed at its base, and the tip end having been cut off, reimplanted by its distal end. The proximo-distal axis was thus reversed. The results show that in most cases there is not only complete determination of the arrangement of the cross section but the parts are already determined proximo-distally. Thus in some cases a shank and tarsus develop toward the more proximal (implanted) end of the graft, while a rudimentary pelvis is formed distally and from this, symmetrical with the graft, a mirror image limb is regenerated. Again there may be an additional limb regenerated from the host, which may or may not be fused with part of the appendage regenerated from the implanted end of the graft. In only three cases, according to GRÄPER, was there evidence that proximal part of the graft was directly converted into the distal part of the appendage and *vice versa*. Nevertheless, the proximal cut surface by a process of regeneration may form a member with reversed asymmetry, by reversing its direction of outgrowth much as in "*Bruchdreifachbildung*". In several cases, however, GRÄPER finds that the polarity of the implanted limb is not fixed, i. e., the limb is in a state that he terms "*reine Querschnittsdetermination*".

GRÄPER's results show that the various parts of the limb bud are already localized, and that the polarity of the limb as a whole is conditioned upon this localization. Beyond this conclusion they do not seem to warrant our going, and it is desirable to inquire further regarding the nature of this polarity, particularly whether there is any evidence for a proximo-distal polarization of the intimate structure of the limb before this localization sets in. The present paper throws some light on this question.

We might now pass to the consideration of the new experiments, were it not for the fact that PRZIBRAM ('24) has recently called into question the whole previous interpretation of my experimental results. He takes exception first to the terminology particularly that used with reference to the axes, saying (op. cit. p. 605): „Dabei ist weder auf die Krümmung des Embryos noch die Wachstumsrichtung der sich

entwickelnden Extremität Rücksicht genommen. Diese rein geometrische, weder tiermorphologisch, noch entwicklungsphysiologisch gerechtfertigte Bezeichnung der Achsen, der Knospe und sohin auch der Transplantate hat leider zu einer Auffassung der Resultate geführt, die mit allem bisher Bekannten in Widerspruch gerät." In order to set the matter aright, i. e., to bring the results into complete subordination to his conception of "*Bruchdreifachbildung*", PRZIBRAM has attempted to elucidate them by a series of models with new axial designations, which, however, show a total misconception of the development of the vertebrate appendage.

It is greatly to be regretted that this misunderstanding should have arisen, for there is much in PRZIBRAM's ('21) conception of polarity and regeneration with which I find myself in agreement, and there seems to be no very essential difference in our views. PRZIBRAM has, in my opinion, simply laid too much stress on one particular axis, and this one he has identified incorrectly. It will therefore be necessary to discuss axes and the normal development of limbs before proceeding.

II. The normal development of the Urodele limb and its axial relations.

It should be stated at the outset that the axes here used have been taken mainly as a system of coördinates to which distances and directions might be referred. Such systems are chosen mainly for convenience and do not necessarily imply deeper significance, though, as it happened here, there does seem to be something fundamental involved. In the present case the system that is most in vogue for bilateral organisms was chosen. Leaving out of account the anterior part of the cephalic flexure and certain controversial points regarding the true morphological axis of the central nervous system, the antero-posterior (cephalo-caudal) or longitudinal axis of the vertebrate embryo may be taken as a line, often curved, joining a point over the end of the fore-brain and the tip of the tail (Fig. 10, $A'P'$)[1]. The dorso-ventral or vertical axis (DV) is taken as a line intersecting the former at right angles at some arbitrary point and intersecting also the dorsal and ventral mid lines. The transverse or medio-lateral axis intersects the other two axes at right angles and passes through the body wall at corresponding points on each side of the body. The three axes of the limb bud are respectively parallel to three axes of the body taken as a whole[2]. The fact that during a certain period of its

[1]) The writer is greatly indebted to Frl. LISBETH KRAUSE for the illustrations to the present paper.

[2]) Curiously enough PRZIBRAM ('24, p. 620) accepts this very same termino-

development the main or longitudinal axis of the body is curved, does not alter its serviceableness as an axis, for the direction of this curved axis, at the point where it is assumed to intersect the other two, is given by the tangent to the curve at that point; and since the curvature within the limb region is negligible, the tangent itself may be taken as the longitudinal axis, the dorso-ventral axis being perpendicular to it at the point of contact. Owing to this curvature, the axes chosen for the grafts placed in normal location (Figs. 10 and 11 orthotopic) are not parallel to those for the abnormal location (heterotopic), but this is immaterial, since the relation of the transplant to that of the surrounding parts, as far as direction is concerned, is the same in both cases.

It would of course be possible to choose some other system of coördinates, but in the present case no other seems to have any special advantage, though all have some disadvantage or other. One difficulty which might arise in connection with the present system is the doubt about the designation of the dorso-ventral and medio-lateral axes in the case of organs that lie on or near the surface of the embryo and somewhere between the median lines and the lateral mid line. This, however, does not enter into consideration in the present connection because the limb in its incipiency lies almost vertically in the body wall about half way between the dorsal and the ventral mid lines (Fig. 3). PRZIBRAM's models show the limb bud as on the dorsal surface of the embryo, a position at variance with actuality.

When we speak of reversal of the dorso-ventral axis of the limb bud when implanted on the same side of the body (homopleural dorso-ventral) this simply means that the limb is rotated 180° around its transverse or medio-lateral axis, i. e., around its center. Grafts from the opposite side of the body are rotated 180° either around the antero-posterior longitudinal axis, in which case the dorso-ventral axis is reversed, as in the heteropleural dorso-ventral implantations, or around the dorso-ventral axis, in which case the antero-posterior direction is reversed, as in the heteropleural dorso-dorsal implantations. In the one

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logy when used by BRANDT. PRZIBRAM bases this discrimination on the supposed difference in cervical flexure between the embryo of *Triton taeniatus* and that of the Axolotl, which he evidently assumes incorrectly to have been the form used by me. A glance at the figure of an embryo of *T. taeniatus* in the tail-bud stage given by BRANDT ('24, p. 545, Skizze 3) shows that this embryo has even a greater flexure than the embryo of *Amblystoma punctatum* shown in Fig. 10. The embryo of the Axolotl, like that of other varieties of *A. tigrinum*, has a curvature slightly greater than that of *T. taeniatus*, though not sufficiently greater to be of any significance even if this form did enter into the present discussion.

case, to use BRAUS's mode of description, it is as if the limb bud were lifted over the mid line, always keeping the inner surface toward the body of the embryo, while in the other it is as if the graft were similarly taken around the front of the head. These are definite manipulations, about the meaning of which there can be no doubt, and the terms used to describe them are exact and appropriate, whatever be the direction of outgrowth of the limb or whatever the body curvature.

Nothing is done in these manipulations to change the actual condition around the border of the graft. It must be borne in mind that the disc of tissue transplanted is a piece of the body wall. It is cut out and fitted into a hole in the body wall of exactly the same size. The wound heals by first intention, and, with good technique, no scar is left. A piece of the body wall has merely been turned around. The rim of each sector of the wound is the same, except that the dorso-anterior portion is thicker than the ventro-posterior. This, however, hardly has any noticeable effect on the smooth healing of the graft. There is no free end ("freies Ende") in these experiments, as PRZIBRAM[1]) maintains. There is no such thing until the limb actually begins to sprout in a direction away from the body wall altogether.

The designation of the radii of the limb bud according to their subsequent morphological relations was deliberately avoided in my previous papers because of the uncertainty of these relations. Even with the results of SWETT's work ('23) on the prospective significance of the material lying on each of the four main quadrants of the limb bud, which were not available when my previous paper on symmetry was written, there is still some doubt as to the exact position, in the disc or bud, of the two main borders (radial and ulnar) and the two main surfaces (extensor or dorsal[2]) and flexor or palmar). One thing is certain, however, the relations shown in PRZIBRAM's models are certainly incorrect, as will appear from a consideration of the mode of development of the limb.

The vertebrate limb is an outgrowth of the body wall, projecting at first laterally. Morphologically two borders, an anterior or preaxial, which corresponds to the radial border of the tetrapod limb, and a posterior or postaxial, which corresponds to the ulnar border, are commonly distinguished. The upper surface, facing dorsally in relation to the embryo, becomes the extensor surface of the limb, and the

[1]) Op. cit. p. 610.

[2]) The use of the word dorsal (or dorsum) as applied to the hand does not mean that this surface necessarily corresponds in all cases to the dorsal surface of the body, though it does according to conventional morphology.

lower surface, turned ventrally, becomes the flexor surface (Fig. 1). In the elasmobranchs this primitive position is usually maintained, and in the embryo the preaxial and postaxial borders are clearly marked by an ectodermal fin fold, continuous around the distal border of the fin-rudiment. In teleosts the fins, especially the pectoral, are frequently rotated away from this primitive position.

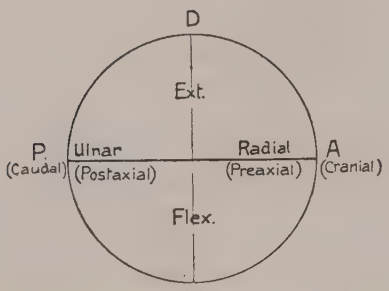

Fig. 1.

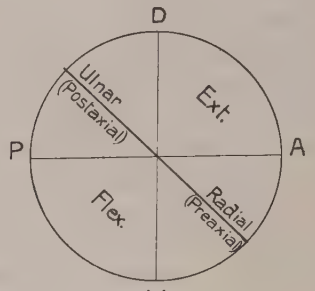

Fig. 2.

Fig. 1. Diagram showing the supposed prospective significance of the four quadrants of the limb bud in the primitive or original position. The *Amblystoma* fore-limb is already beyond this stage, if it ever passes through it, when the operations are done.

Fig. 2. Same, after supposed torsion. This gives approximately the supposed axial relations at the time of operation.

In the tetrapod limb the borders, preaxial and postaxial, are often not readily distinguishable, particularly in the amphibians, where the limb develops as a cylindrical outgrowth, with a rounded tip, without any ectodermal fold to mark them. In the lizards, however, such an ectodermal fold is present, as has been described and figured by MOLLIER ('95), PETER ('02) and by BRAUS ('04). This fold, as can readily be observed, is longitudinal, the preaxial portion of it being anterior, the postaxial posterior. Soon a torsion takes place, which brings the preaxial or radial border to a nearly ventral position and the ulnar or postaxial dorsal. When these borders are first recognizable in the fore-limb of *Amblystoma punctatum*, they are seen to be about half way between the assumed original and the twisted position, as may be realized from the plane of flattening of the hand which is inclined about 45° to the horizontal plane of the embryo. Since, however, there is no means of identifying these borders at an earlier stage, it is not possible to determine directly which radius of the limb disc represents the future radial border. SWETT's work ('23) indicates, however, that the material which is distributed along the proximal part of the radial border is found in the antero-ventral quadrant of the limb bud not far from the center and about on a radius which lies from 30° to 45° anteriorly from the dorso-ventral

axis (Figs. 2, 6 and 7). At the time of grafting the material composing the limb bud of *Amblystoma* is, therefore, already much nearer to the definitive location than it is in such forms as the lizard, where considerable torsion occurs after the limb bud becomes visible. After the *Amblystoma*-limb has grown out to some length and the digits are forming, further torsion takes place, which makes the radial border ventral and the ulnar dorsal.

When the limb bud begins to grow it is merely a nodule in the body wall. Its primary direction of growth is away from the mid line, almost in the transverse axis (Figs. 3 and 4). However, owing to the curvature of the surface of the yolk the axis of rotation (*ML*) is slightly inclined to the transverse (*R't-L't*). A further complication arises from the fact that the limb does not remain for any length of time in an extended position, but inclines gradually posteriorly and continues its growth as adducted. This is beginning to show even in the stage represented in Fig. 4, and by stage 39 (Fig. 5, *Pr—Di*) the angle with the transverse axis, projected on a horizontal plane is about 45°. Later, for a time, it becomes pressed almost flat against the flank. Seen from the side the limb does not point directly posteriorly but dorsally about 30° to the horizontal.

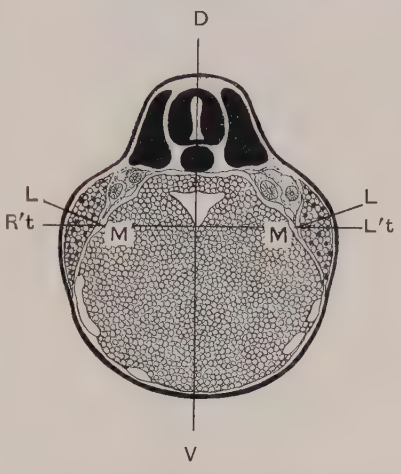

Fig. 3. Cross section through the fore-limb region of an *Amblystoma* embryo (stage 36), showing the position of the limb bud in the body wall and the relation of the dorso-ventral (*D V*) and transverse axes (*R't—L't*). The proximo-distal or medio-lateral axis (axis of outgrowth) of the limb (*ML*) is inclined at a small angle to the transverse.

In the diagrams illustrating my previous paper and in many of the figures of the actual specimens, the developing limbs are viewed from the side, and hence appear as if projected on the median plane of the embryo. In these figures the lateral growth is, therefore, not shown, but it is clearly represented in the figures showing dorsal or ventral views, and it was taken for granted that this would be understood. Unfortunately, however, PRZIBRAM (op. cit. p. 605—6) has assumed in his discussion that the actual, not the projected, direction of outgrowth is posterior or dorso-posterior and that the growing point lies on the periphery of the limb disc. He, accordingly, inserts in his modification of my diagram the designations, *Di* (distal) and *Px* (proximal) in place respectively of *P* (posterior) and *A* (anterior),

and imagines, therefore, that rotating the limb disc 180° merely reverses the direction of outgrowth[1]). This is the main source of the confusion in his discussion, as will be seen more fully below.

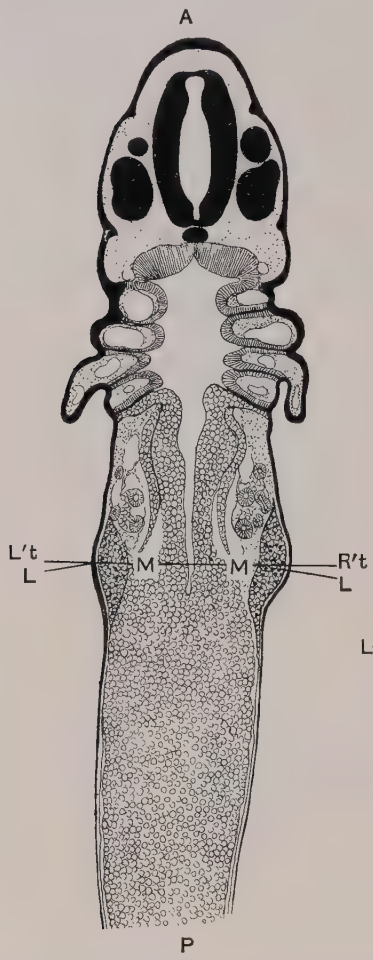

Fig. 4.

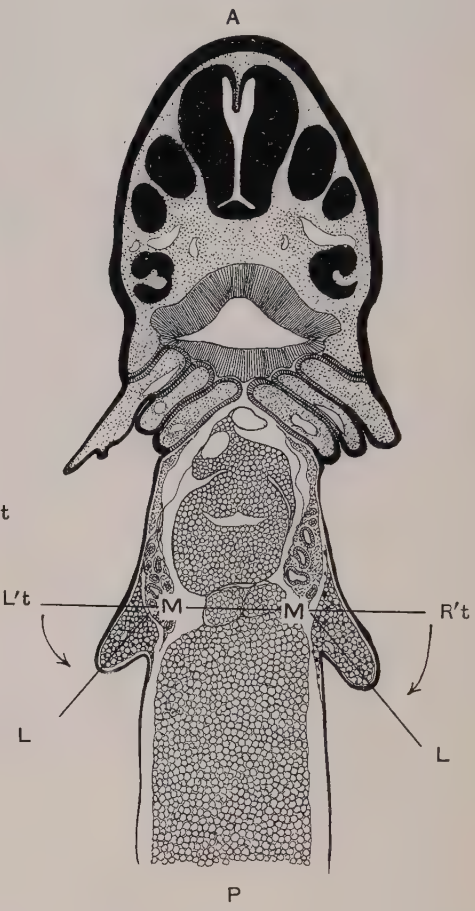

Fig. 5.

Fig. 4. Horizontal or frontal section through the anterior end of an *Amblystoma* embryo, similarly showing the axial relations of the limb bud. In this plane also the proximo-distal axis (*ML*) is already beginning to show its inclination to the transverse (*R't*—*L't*).

Fig. 5. Frontal section through the anterior end of an *Amblystoma* embryo in stage 39, showing the greater inclination (ca. 45°) of the proximo-distal axis (*ML*) to the transverse (*R't*—*L't*).

We may now attempt to locate more precisely the borders and surfaces of the definitive limb in the disc of tissue out of which it develops. Assuming them to be in the same relative position at the

[1]) Op. cit. p. 608.

stage at which the operations are done as later, when these features first become visible, an oblique line (Figs. 2 and 6) crossing the disc through the dorso-posterior and ventro-anterior quadrant would pass through the ulnar and radial borders and hence divide the flexor from the extensor surface. The only reliable information bearing on this question is that given in SWETT's ('23) paper. By transplanting

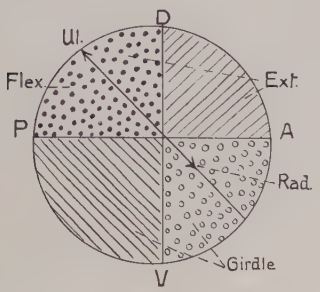

Fig. 6.

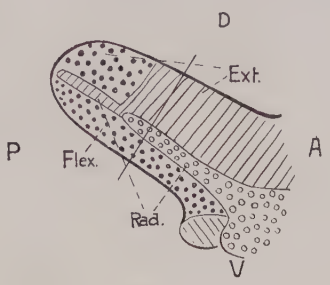

Fig. 7.

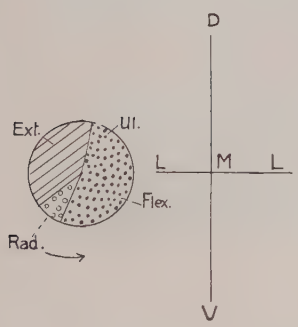

Fig. 8.

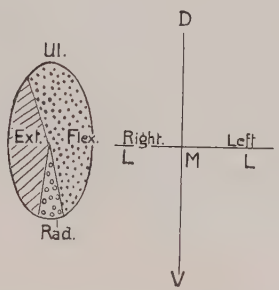

Fig. 9.

Fig. 6—9. Diagrams to show axial relations and localization of material in the limb of *Amblystoma*, based upon figures taken from SWETT. The four quadrants of the bud (Fig. 6) are differently shaded to show the distribution of the material in each to the parts of the definitive limb (Fig. 7), both viewed from the right side. *A*, anterior; *D*, dorsal; *P*, posterior; *V*, ventral; *Ext.*, extensor surface or dorsum of arm; *Flex.*, flexor or palmar surface; *Rad.*, radial border; *Ul.*, ulnar border; *DMV*, dorso-ventral axis of embryo; *LML*, transverse axis of embryo seen in transverse section.

Fig. 6. Limb bud at time of transplantation viewed from the side.

Fig. 7. Limb, about stage 40.

Fig. 8. Limb about stage 40 seen in cross section and in relation to the axes of the embryo before torsion; adduction and flattening of hand are incomplete.

Fig. 9. Limb bud in cross section and in relation to the axes of the embryo after complete torsion, adduction and flattening of hand.

various halves of the limb bud after staining the mesoderm *intra vitam*, it was found that the posterior half does form the whole of the flexor surface and extends over the tip of the limb, forming the distal part of the extensor surface as well. The anterior half, on the other hand, forms the basal portion of the extensor surface and a strip running

out toward the tip on the radial border. Taken by quadrants, the dorso-posterior sector forms the whole flexor surface and the distal portion of the extensor, the dorso-anterior sector forms the rest of the extensor surface and extends along the radial border almost to the tip. The ventro-anterior portion forms largely the shoulder but sends out a strip of cells along the radial border, while the posterior sector does not participate in the formation of the free limb at all but enters only into the shoulder and body wall. This is shown diagrammatically in Figs. 6 and 7. If, instead of dividing the limb bud vertically and horizontally, as in SWETT's experiments, it were split obliquely at the proper inclination, the cut would no doubt pass through the growing point of the limb and separate the flexor from the extensor surfaces.

From the above it is seen that turning the limb bud 180° does not reverse proximal and distal portions, as PRZIBRAM maintains, except in so far as the growing point is somewhat eccentric to the disc as a whole, but it does bring the material that was to have formed the radial border into the position of that which normally forms the ulnar and *vice versa*. Likewise the position of flexor and extensor portions is interchanged.

Limbs which are thus rotated also begin their growth in a lateral direction, i. e., away from the mid line; their primary direction of outgrowth is not changed. It is only when the growth axis becomes inclined or adducted that the effect of the rotation becomes apparent. Then it is seen that the limb, when viewed from the side, points anteriorly instead of posteriorly in accordance, approximately, with the rotation imparted to the graft. It soon shows, however, the effect of its unusual position by becoming inclined slightly dorsally, whereas it should point ventro-anteriorly, were it entirely uninfluenced by the change in its relations to the surrounding parts of the embryo. After the axis of outgrowth becomes inclined or adducted anteriorly toward the body it is seen that the surface turned toward the body and ventrally is to form the flexor surface. However, not the radial but the ulnar border is formed dorsally, showing that the materials entering into these parts of the limb are not yet fully determined at the time of operation. In other words, no matter how the limb is adducted, whether anteriorly or posteriorly, the radial border is ventral and away from the body and the ulnar is dorsal and toward the body (Fig. 8). The first visible effect of the reversal of the antero-posterior axis is a change in the direction of inclination or adduction of the growing bud. This accords approximately with the imposed rotation of the graft, and the relation is apparently irreversible. On the other hand the borders of the hand and arm are formed in accordance

with their orientation with respect to the dorso-ventral axis of the body, and are not affected by their relation to its antero-posterior axis, i. e., not by the direction of adduction. Were the polarity of both axes of the bud already determined, as PRZIBRAM maintains, we should expect the same results in *Amblystoma punctatum* as in *Triton taeniatus*, where, according to BRANDT, inversion of the graft produces an inverted limb. Yet the latter never occurs in *Amblystoma*, except when a ring of tissue surrounding the limb bud is rotated, as shown by NICHOLAS ('22). These facts are quite inexplicable on PRZIBRAM's hypothesis, and a difference in the amount of cervical flexure, even if it did exist to a considerable degree, would have nothing to do with the case.

Were the materials so localized in the limb bud that the radio-ulnar axis coincided exactly with the dorso-ventral axis of the bud, and were the growing limb to be adducted exactly in a horizontal plane, then we might say that after inversion of the antero-posterior axis of the bud, the material that was originally destined to form the flexor surface still did so, though owing to the rotation it is now located in the anterior half of the disc instead of in the posterior. At the same time the radial border would remain ventral, though developing out of a portion of the disc that was originally dorsal. We then might say that the extensor-flexor relation were fixed in the bud, but the radio-ulnar determined in relation to the surroundings. Unfortunately, however, the relations are not so simple as this. The dorso-ventral axis of the bud does not correspond exactly to the radio-ulnar axis of the limb, and the amount of torsion that takes place in both the normal and the grafted limbs during the process of adduction is uncertain, and probably in the opposite direction in the two cases. This is seen first from the direction of pointing, which, when the bud is rotated 180°, is not 180° removed from the normal, and secondly from the fact that the hand of the limb derived from an inverted bud, like the normal hand, lies at first with its radial border ventro-lateral, instead of ventro-medial as it would were the ulnar and radial borders merely interchanged by the rotation. Thus it is not possible at present to know where the material for, e. g., the radial border is located after reversal of the limb bud. While it is probably not 180° removed, it cannot be less than 90° from its original position. The same uncertainty holds for all sectors of the limb alike. All that is absolutely certain, is that the distribution of the material in the limb bud is different with each different orientation. It is likely that, with sufficient patience, many of these questions could be settled by the stained-mesoderm method, which SWETT has applied successfully to the study of the normal limb. Pending such

an investigation one's avoidance of any but a geometric system of axes may be considered pardonable.

One of the most interesting circumstances connected with the peculiar axial relations set forth above is that they occur in a system that is equipotential in the sense defined by DRIESCH ('02). PRZIBRAM has called this into question (op. cit. p. 608), citing SWETT's work as showing that the localization of parts in the limb bud is already determined. In taking this position PRZIBRAM fails to take account of DRIESCH's distinction, now almost universally accepted, between "prospective potency" and "prospective significance". SWETT has shown that certain portions of the limb bud in normal development form certain definite portions of the limb. In other words, their prospective significance has been ascertained. My own experiments show, nevertheless, that all four of the cardinal halves of the limb bud are totipotent. Taken with other evidence given in my previous paper, this constitutes a rigorous proof of equipotentiality. It is true, however, that the limb bud used in the experiments (three and a half somites in diameter) includes some material that ordinarily does not enter into the make-up of the limb. This material may, nevertheless, form a limb if given the opportunity. It varies as to the intensity with which this power acts, as shown by differences in time of development and in the percentage of cases with complete development. It is all totipotent but is equipotential only as measured by the end result, and not as measured by the velocity of development. In other words, there is a gradient of some kind in the limb bud, with a growth center, having the highest potential, which passes over gradually through zones having a lower potential (though still totipotent), into regions where the potency to form a limb is altogether absent.

Further experiments to test the question of totipotentiality have been made, but will be reported later and require only brief mention here. By using rectangular instead of semicircular grafts it is possible to halve the limb and interchange the two pieces without reversing their orientation. The result of such experiments, provided the pieces are not too wide antero-posteriorly, is the development af a normal limb, showing, when taken in conjunction with the experiments of rotating the limb bud, that it is not the transposition of the material of the two halves but the reversal of the orientation of the antero-posterior axis that brings about the reversal of the limb.

III. Experimental.

As already pointed out, the reversal of the medio-lateral axis of the limb bud, the axis of outgrowth, can be accomplished only by stripping off the ectoderm and replacing the mesoderm inside out.

The earlier experiments[1]) in inoculating limb mesoderm were done mostly with embryos in stage 29—31 (Fig. 10), at which period the cells of the limb bud hang only loosely together and are easily disarranged. Later it was found that in slightly more advanced stages (33—34) (Fig. 11) the limb mesoderm holds more firmly together, making it possible to transplant it without much injury and at the same time to control its orientation.

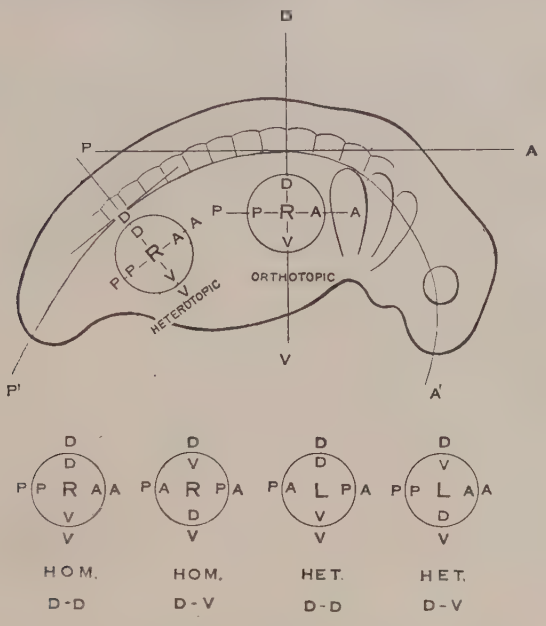

Fig. 10. Diagram showing the eight different operations included in the earlier experiments, in which the medio-lateral or proximo-distal axis remained unchanged. The outline of an *Amblystoma* embryo, stage 29, is shown above. The circles within it represent the limb bud, in the normal (orthotopic) and the abnormal (heterotopic) location. The four circles below represent the four different ways in which limb buds may be oriented with reference to the cardinal points of the embryo; the letters (*D*, dorsal; *V*, ventral; *A*, anterior, and *P*, posterior) within the circles designate the original cardinal points of the transplanted limb, those outside the corresponding points of the embryo. The operations are represented as on the right side. *R*, right limb bud; *L*, left limb bud; *hom.*, homopleural; *het.*, heteropleural.

The figure has been slightly modified from its original form so as to show the curvature of the actual antero-posterior axis (*A'P'*) of the embryo. The geometrical axes used in orienting the graft are respectively the tangent and the perpendicular to the curved axis where the plane of the dorso-ventral axis of the limb bud cuts it.

In the first of the latter experiments made in 1920 the following method of operation was used. A glass tube, drawn out to a capillary point and filled with salt solution, was inserted under the skin of the flank of the host dorsal to the pronephric duct and pointed ventrally in the region of the ninth or tenth somite. By blowing into a rubber

[1]) Cf. Harrison ('18).

tube connected with the glass the salt solution was forced out under the skin, lifting the latter from its substrate and making a large pocket for the reception of the graft. The ectoderm of the fore-limb region of another embryo was similarly raised, and after its removal, the limb mesoderm, including in most cases the pronephros, was cut out by a circular incision three and a half somites in diameter (from the third to half way trough the sixth). While the edges of the graft con-

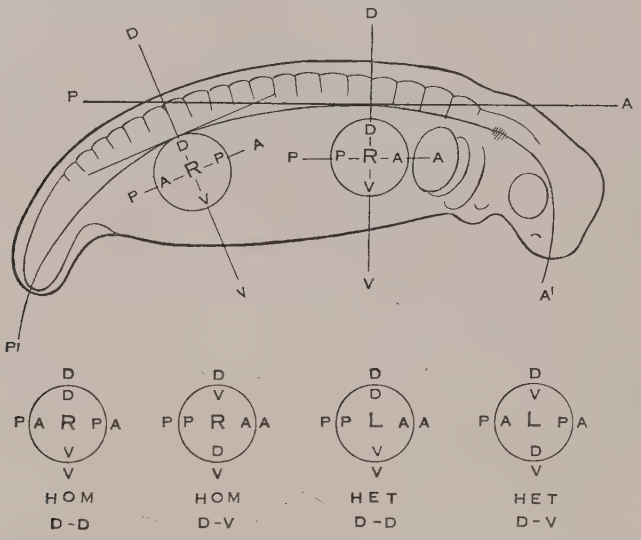

Fig. 11. Similar diagram, showing the different combinations when the mesodermal limb mesoderm is grafted with its original medial surface turned toward the skin. An older embryo (stage 33) is shown. Actually only the heterotopic operations were carried out in this case. Note that the combinations in which the dorso-ventral and antero-posterior axes occur in the two figures are different.

tracted and curled up to some extent, often with loss of material, the main mass remained solid and could be transplanted to the pocket in the flank of the other embryo without difficulty. Its orientation could be readily controlled by the position of the pronephros in it or by differences in the thickness and pigmentation of the different sectors.

The grafts were all placed with the medial surface facing outward or toward the ectoderm. It was not thought necessary to do control experiments with the lateral surface of the graft turned toward the skin, since the previous experiments with the whole bud, mesoderm and ectoderm, were thought to be a sufficient control.

Only heterotopic transplantations were made because of the practical impossibility of freeing the wound bed in the limb region from limb forming cells without irreparable injury to the ectodermal pocket. There is no disadvantage, however, in this limitation, for the hetero-

topic transplantations are less involved with secondary disturbing factors than the orthotopic.

Taking grafts from both sides of the body and placing them with the dorso-ventral axis either in normal or inverted direction, four orientations are possible, just as in the previous experiments in which the medio-lateral axis was left unchanged. There is this difference, however. The combinations of axial directions are different in the two series, as shown in Table I.

Table I. Showing the possible combinations of axial relations in both the previous experiments (lines 1, 3, 5, 7) and in the present ones (lines 2, 4, 6, 8).

Side of origin of graft	Orientation of axes of graft with respect to host			Abbreviated designation
	Dorso-ventral or vertical axis	Antero-posterior or longitudinal axis	Medio-lateral (proximal-distal) or transverse axis	
Homopleural	dorso-dorsal	antero-anterior	medio-medial	Hom. dd, aa, mm
Homopleural	dorso-dorsal	antero-posterior	medio-lateral	Hom. dd, ap, ml
Homopleural	dorso-ventral	antero-posterior	medio-medial	Hom. dv, ap, mm
Homopleural	dorso-ventral	antero-anterior	medio-lateral	Hom. dv, aa, ml
Heteropleural	dorso-dorsal	antero-posterior	medio-medial	Het. dd, ap, mm
Heteropleural	dorso-dorsal	antero-anterior	medio-lateral	Het. dd, aa, ml
Heteropleural	dorso-ventral	antero-anterior	medio-medial	Het. dv, aa, mm
Heteropleural	dorso-ventral	antero-posterior	medio-lateral	Het. dv, ap, ml

Out of the twenty-one experiments made in 1920 only two limbs developed. In fourteen cases the grafts were resorbed, and five individuals died. This discouraging result led to the temporary abandonment of the study, but in 1925 the experiments were again taken up and a somewhat different technique followed. The subepidermal pockets were made with a needle instead of by hydraulic action. While the mortality was heavier, owing to one particular bad bunch of eggs used on certain day, the percentage of resorbed grafts was much lower. There were seven positive cases and nine resorptions. Why there should be this difference is not clear, but it is thought that the pocket made by the capillary tube method was in most cases beneath the mesoderm, whereas when made by the needle mesoderm and ectoderm were usually separated.

The number of positive cases is small — only nine in all, but all four orientations are represented, and the results are wholly consistent within themselves and with the results of the previous experiments, in which the medio-lateral axis was not turned. Furthermore there were no complications due to reduplication except in a single case. The results show that the limb buds are no more affected by the

reversal of the medio-lateral axis than by that of the dorso-ventral. They do not grow into the body, but grow out free from it. They become adducted or "point" in accordance with the imposed direction of their antero-posterior axis and their ultimate asymmetry or laterality is determined by this factor acting in relation to the orientation of the surrounding parts. Table II summarizes the results.

Table II. Transplantation of fore-limb mesoderm with the original medial surface turned toward the ectoderm, the medio-lateral axis being thus reversed. Summary of results.

Orientation of graft	Resulting limb						
	Single limb asymmetry reversed	Single limb not reversed	Double limb	Indeterminate	Abortive or resorbed	Dead	Total
Hom. dd, ap, ml	2				3	1	6
Hom. dv, aa, ml		2			6	4	12
Het. dd, aa, ml	3*		1		4	5	13
Het. dv, ap, ml		1		1	9	5	16
Total	5	3	1	1	22	15	47

* Including one case with defective (styliform) hand.

Table III, taken from a previous paper but modified in form and showing the results of experiments in which the limb bud was transplanted to the flank without reversal of the medio-lateral axis, is given for the sake of comparison.

Table III. Transplantation of fore-limb bud, including covering ectoderm, the medio-lateral axis being left unchanged. Summary of results.

Orientation of graft	Resulting limb						
	Single limb asymmetry reversed	Single limb not reversed	Double limb	Indeterminate	Abortive or resorbed	Dead	Total
Hom. dd, aa, mm		3	4	3	2	7	19
Hom. dv, ap, mm	11		1		14	5	31
Het. dd, ap, mm		8	2	1	12	5	28
Het. dv, aa, mm	7	1 (?)*	8	8	16	20	60
Total	18	11 (12?)	15	12	44	37	138

* There is evidence that this case involved an experimental error.

Histories of individual cases show that the development of the grafted tissue follows an approximately normal course, except as modified by the orientation of the antero-posterior axis. Just as was found in previous experiments there is, however, greater variation in the posture of the growing limb in heterotopic than in orthotopic grafts.

1. Limb bud grafted on the same side of the body, the antero-posterior but not the dorso-ventral axis being inverted (Hom. dd, ap, ml).

The grafted tissue is here turned 180° about its dorso-ventral axis. There are two positive cases in the group with essentially similar histories, each showing a left limb on the right side (disharmonic or *wirtsseitenverkehrt*).

Experiment Tr.M 20. Operation on the right side; both, donor and host, in stage 34; skin pocket made with capillary tube. Six days after the operation a small limb but was noted pointing anteriorly. Eight days later this had developed into a bidigitate limb, which pointed anteriorly and slightly laterally, with the palmar surface turned medio-ventrally. Six days after this the fore-arm and hand had become well differentiated. The third digit was distinct and the fourth showed as a nodule on the ulnar border. Figs. 12 and 13 were drawn from the preserved specimen killed about ten days later at the age of a month dating from the operation.

Fig. 12.

Fig. 13.

Figs. 12 and 13. Transplantation of right limb mesoderm to right side (Hom. dd. ap. ml). The result is a left limb on the right side. Experiment Tr. M 20. ×10.

Fig. 12. Side view of limbs, normal and transplanted (T_2) drawn from specimen, preserved a month after operation.

Fig. 13. Ventral view of same.

The gross findings were verified in transverse sections of the larva. Thus the flexor surface of the hand is that which is turned toward the body. The shoulder girdle is well developed, with scapula, procoracoid and coracoid. Like the free limb it is reversed. The coracoid and procoracoid are prolonged posteriorly instead of anteriorly. The scapula extends to the ventral border of the muscle plates, but there is no suprascapula.

Experiment Tr.M 40. Operation on right side at stage 33; pocket made with capillary tube. This case showed greater delay in the appearance of the limb bud. It is recorded as small and pointing anteriorly

twelve days after the operation. At the time of preservation it hat not developed the fourth digit as distinctly as the previous case, and its posture was slightly different in that it stuck out more sharply to the side.

Transverse sections show that in this case, too, the palm of the hand is turned toward the body and ventrally, but also to some extent anteriorly. The shoulder girdle is reversed and in all respects much like that in the previous case.

Fig. 14.

Fig. 15.

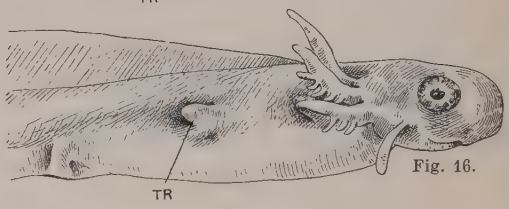

Fig. 16.

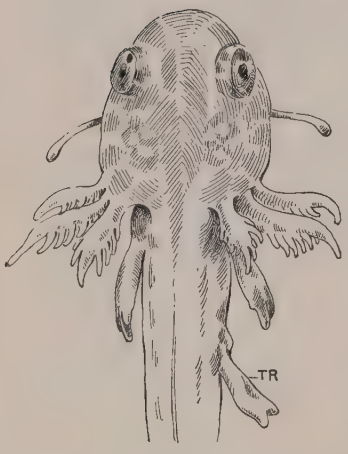

Fig. 17.

Fig. 18.

Fig. 19.

Figs. 14–19. Transplantation of right limb mesoderm to right side (Hom. dv, aa, ml). The result is a normal right limb (T_r) on the right side. Experiment Tr. M. 41. ×10.

Fig. 14. Embryo five days after operation, dorsal view. Stage 39+.
Fig. 15. Same, lateral view, the grafted limb (T_r) points dorso-posteriorly.
Fig. 16. Six days after operation, lateral view. Stage 40.
Fig. 17. Thirteen days after operation, dorsal view. Stage 45.
Fig. 18. Thirteen days, view from side and slightly from above.
Fig. 19. Twenty days, lateral view.

2. Limb bud grafted on the same side of the body, the dorso-ventral but not the antero-posterior axis being inverted (Hom. dv, aa, ml).

In this operation the limb mesoderm is turned 180° about its antero-posterior axis. The group also includes but two positive cases with essentially similar histories, both with normal harmonic (*wirtsseitenrichtig*) limbs.

Experiment Tr.M 41. Operation on the right side at stage 33+; pocket made with needles. Five days after the operation the grafted bud is recorded as pointing distinctly dorso-posteriorly (Figs. 14 and 15) and the next day it had grown materially (Fig. 16). Seven days later, with the larva at stage 45 (Figs. 17 and 18), the grafted limb was found to be in normal posture and about equivalent to the normal limb in stage 43. When preserved, at twenty days, the third digit had begun to elongate and the fourth showed as a prominence on the ulnar border of the hand (Fig. 19). The elbow is normally bent, the palm turned toward the body and ventrally (verified later in sections). No movements were observed.

Sections show the shoulder girdle to be well developed and normally oriented. Scapula, coracoid, and procoracoid are all present but there is no suprascapula.

Experiment Tr.M 43. Operation on the right side a stage 33+; pocket made with needles. This case differed from the above solely in showing the hand lying at first in an almost horizontal plane, with the palm downward. Later it twisted itself more nearly into normal posture, but it remained closer to the horizontal than in the previous case.

The shoulder girdle is seen in cross sections to be normally oriented, with well developed scapula and a coracoid extending anteriorly from the glenoid cavity. There is only one process — no separate coracoid and procoracoid. The palmar surface of the hand, as above described, was also seen in the sections to face ventro-medially.

3. Limb bud grafted on the opposite side of the body, both dorso-ventral and antero-posterior axes being directed normally (Het. dd, aa, ml).

In this experiment the limb bud is placed as if it were taken from one side of the body and moved across to the other without rotation in any direction.

Four positive cases constitute the group, one with a double hand and one with a defective styliform appendage. The primary member of the duplicity agress with the other three cases in having its original prospective asymmetry reversed.

Experiment Tr. M 45. Limb bud moved from right side to left, stage 33+; pocket made with needles. Five days after the operation

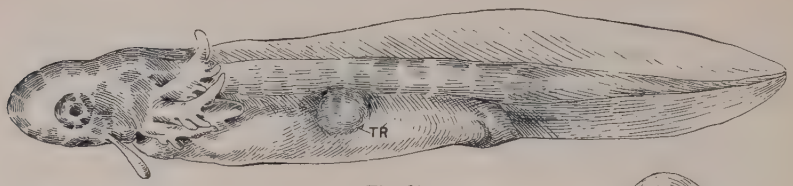

Fig. 21.

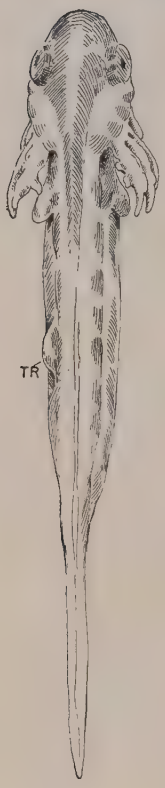

Fig. 20.

Fig. 24.

Fig. 22.

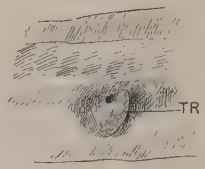

Fig. 23.

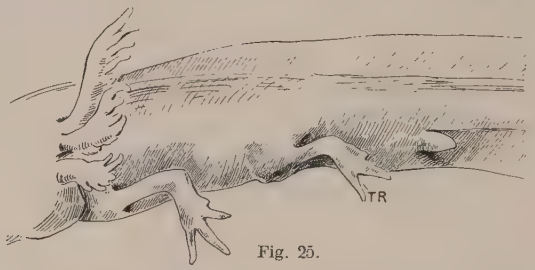

Fig. 25.

Figs. 20—25. Transplantation of right limb mesoderm to left side (Het. dd, aa, ml). The result is a normally oriented left limb (*Tr*). Experiment Tr. M. ×10.
Fig. 20. Embryo five days after operation. Stage 39.
Fig. 21. Same from side.
Fig. 22. Nine days, dorsal view. Stage 42.
Fig. 23. Side view of grafted limb, nine days.
Fig. 24. Thirteen days.
Fig. 25. Lateral view, nineteen days.

the grafted bud was distinct, pointing slightly posteriorly (Figs. 20 and 21). Three days later it is recorded as pointing dorso-posteriorly at an angle of ca. 45°. During the next three days (Figs. 22 and 23) the limb bud turned further dorsally, until it became nearly vertical. About this time the interdigital notch appeared, the limb being between stages 40 and 41 and the host at stage 44. As the limb developed further, it gradually returned to a normal posture (Figs. 24 and 25). Twenty days after the operation the specimen was preserved. The elbow and hand were then well developed and the limb in almost the exact posture of the normal left limb and only slightly behind it in development (Fig. 25).

Sections show the palm of the hand facing medio-ventrally, more nearly vertical than horizontal. The shoulder girdle in this case is the largest and best developed of all examined. It is normally oriented and all of its constituent parts readily identifiable. The suprascapula alone is lacking.

Experiment Tr. M 44. Right to left; operation at stage 33+. In this case the limb bud grew out posteriorly and did not rotate dorsally. It remained closely adducted and developed into a normally formed left limb. When preserved, twenty days after the operation, the third digit was beginning to elongate and the fourth was only faintly visible. The palm of the hand faced the body. This case has not been examined in sections.

Experiment Tr. M 46. Left limb mesoderm to right side; operation at stage 32 (slightly earlier than in the other cases). The early history of this case is essentially similar to that of the two previous ones. Nine days after the operation, when the embryo was a little beyond stage 40, the grafted bud pointed dorso-posteriorly about as a normal limb of stage 38, but more out to the side than usual (Figs. 26 and 27). Seven days later the limb had become fan like and stuck out still more sharply to the side, showing little or no adduction or torsion (Fig. 28). The posterior (primary) member of the complex has two long digits with the beginning of the ulnar digital prominence on the posterior border. The secondary limb, arising on the anterior or radial border of the primary, shows the beginning of the first two digits A week later the elbow had become marked, bending ventrally, and the digits much more definite (Figs. 29 and 30). The first two digits are syndactylous in both the primary and the secondary members. Indications of both the third and fourth digits are present on the primary limb, while the secondary shows only the third. It is a plain case of radial reduplication, the primary member being contra-lateral to its origin, and in harmony with its new surroundings. The limb in this case exhibited slight movement — flexion of the elbow and both anterior and posterior adduction at the shoulder joint.

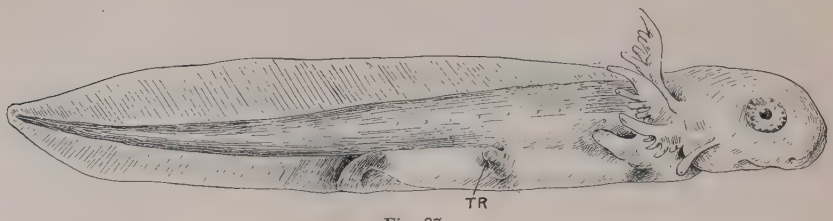

Fig. 27.

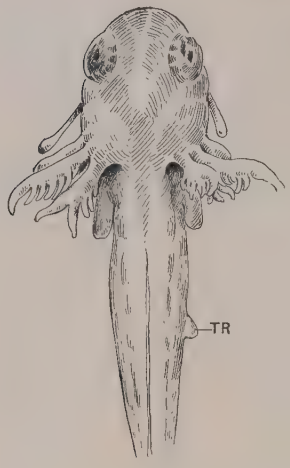

Fig. 26.

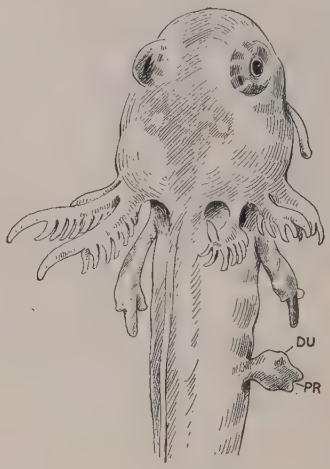

Fig. 28.

Fig. 30.

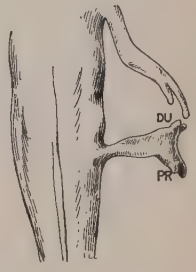

Fig. 29.

Figs. 26–30. Transplantation of left limb mesoderm to right side (Het. dd, aa, ml). The result is a limb with a double hand, the primary member (*Pr*) being a right and the duplicate (*Du*) a left. Experiment Tr. M 46. ×10.

Fig. 26. Embryo, dorsal view, nine days after operation. Stage 41. Grafted limb (*Tr*) points dorso-posteriorly.
Fig. 27. Lateral view, nine days.
Fig. 28. Dorsal view, sixteen days.
Fig. 29. Dorsal view, twenty-three days.
Fig. 30. Lateral view, twenty-three days.

Sections show the shoulder girdle to be defective. There is no scapula, i. e., no cartilage dorsal to the glenoid cavity. Ventral to the articulation there is a single plate of cartilage extending both

anteriorly and posteriorly. There is no certain criterion by which it can be judged right or left except that the shape of the articulation indicates it to be a right. The muscles are well developed. The humerus sticks out directly to the side and is single but rather stout. There are three long bones in the fore-arm, a single common radius in the middle, with an ulna on each side.

Experiment Tr. M 62. Left limb mesoderm to right side; stage 33. The early history of this case was also like the normal. The limb began to point posteriorly, but a little later directed it self more sharply to the side than usual. At twelve days (Fig. 31) it looked as if the interdigital notch were just appearing, but four days later, when the larva had begun to feed, the grafted limb was seen to be defective, ending in a single pointed process (Figs. 32 and 33). The elbow was normally bent, but the upper arm was not free from the body, being held down by skin. The limb was them amputated at the elbow, and two weeks later it had regenerated completely but was again styliform (Fig. 34). In the meantime the upper arm had been freed from the body. The limb was again amputated, this time close to the shoulder. Observed eight days later it was found to be again regenerating as a defective. Unfortunately the larva died a day or two later, and when found was too far decomposed to be of use.

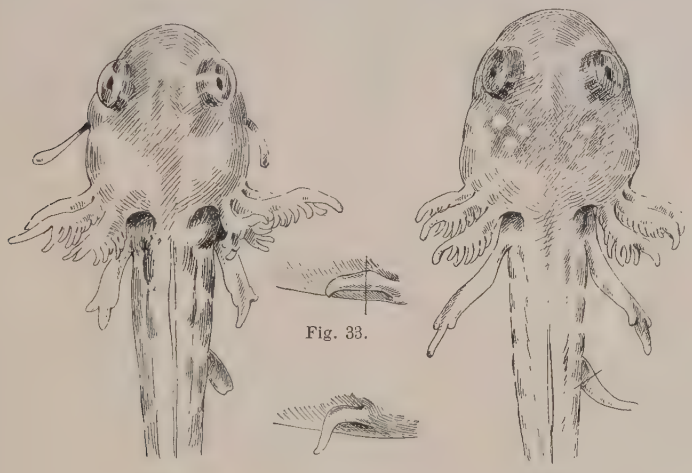

Fig. 31. Fig. 34. Fig. 32.

Figs. 31–34. Transplantation of left limb mesoderm to right side (Het. dd, aa, ml), resulting in a defective right limb. Experiment Tr. M 62. ×10.

Fig. 31. Twelve days after operation. Stage 45.
Fig. 32. Sixteen days, dorsal view. Line shows place of amputation.
Fig. 33. Lateral view of limb, sixteen days.
Fig. 34. Regenerated defective limb, fifteen days after amputation.

4. Limb bud grafted on the opposite side of the body, both dorso-ventral and antero-posterior axes being reversed (Het. dv, ap, ml).

This operation is as if the limb disc were moved across from one side to the other, the inner surface becoming the outer, and then rotated 180° about its center. There is only one positive case in the group, but one other will also be considered.

Experiment Tr. M 48. Left limb mesoderm to right side; stage 34; pocket made with needles. In this case a distinct limb bud was formed, pointing antero-ventrally. However, it never developed beyond a normal limb in stage 39, and was later reduced to a wartlike process attached between the fifth and sixth intercostal grooves.

Sections show that the abortive limb contains a blood vessel and a small round nodule of cartilage. In the body wall there is a plate

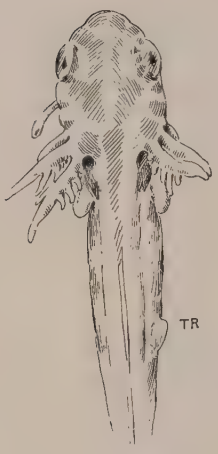

Fig. 35.

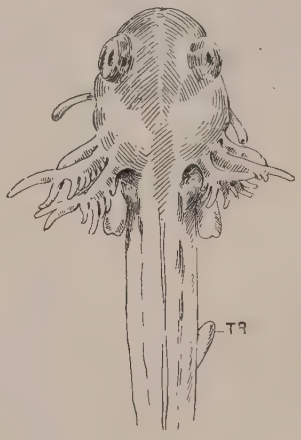

Fig. 37.

Fig. 39.

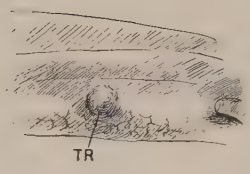

Fig. 36.

Fig. 38.

Figs. 35—39. Transplantation of left limb mesoderm to right side (Het. dv, ap, ml). Result a left limb (*Tr*) on the right side. Experiment Tr. M 63 ×10.

Fig. 35. Seven days after operation, dorsal view; limb points anteriorly.
Fig. 36. Seven days, lateral view. Stage 40.
Fig. 37. Nine days, dorsal view. Stage 42.
Fig. 38. Twelve days, ventral view. Stage 44+.
Fig. 39. Sixteen days, dorsal view.

of cartilage like a shoulder girdle. It is quite extensive, being 0,25 mm long, and posteriorly it runs out into two separate processes like the procoracoid and coracoid in girdles that are reversed. There is also a hook-like process of cartilage attached to the ventral border of the girdle.

Experiment Tr. M 63. Left limb mesoderm to right side, stage 33; pocket made with needles. Seven days after the operation the limb bud was distinct and pointed slightly antero-dorsally (Figs. 35 and 36). Two days later the grafted bud had elongated materially (Fig. 37), but in three days more (twelve days after the operation) it changed very little, reaching ca. stage 40 when the embryo was between stages 44 and 45 (Fig. 38). The transplanted limb then pointed sharply antero-dorsally. Four days afterward the first two digits had appeared and a slight protuberance marked the ulnar border (Fig. 39). The limb stands out stiffly at an angle of nearly 45° to the transverse, with the palm facing the body and ventrally. The larva was weak and never took food, so that development ceased at this point, except that the elbow bend became a little more definite. Although defective as to ulnar digits, the limb is, nevertheless, clearly a left on the right side.

Sections show that the ventro-medial surface of the hand is the palm and the dorso-medial border the ulnar. The humerus is thick and fills the arm, there being little or no muscular tissue. The girdle is indeterminate as regards its right-left relations. A considerable process extends anteriorly from the articulation, but the posterior extension is longer. There is no distinction between coracoid and procoracoid.

IV. Discussion of Results.

In order to compare more clearly the results of the present with those of the previous experiments, Tables II and III have been combined into a single one (Table IV), which shows the asymmetry or laterality of the limb with respect to the side of the host on which it is placed, instead of with respect to its origin. As formerly, those limbs in which the asymmetry corresponds to that of its surroundings (*wirtsseitenrichtig*), as when a right limb grows on the right side, are termed harmonic, while those in which the asymmetry is opposite (*wirtsseitenverkehrt*), as when a left limb grows on the right side, are termed disharmonic. Inspection of the last named table shows that the single limbs, of which there are thirty-eight cases, have their asymmetry determined according to the orientation of the longitudinal or antero-posterior axis. Where this is not reversed (aa) the limb developed is harmonic; where it is reversed (ap) the limb is disharmonic. Neither the dorso-ventral nor the medio-lateral axis is of consequence

in this respect, for both harmonic and disharmonic limbs occur when either one alone or both of these axes together are reversed, as well as when both are normally directed. There is only one exceptional case, one in which a disharmonic limb developed where the antero-posterior axis was not turned. This was commented upon previously[1]) and is probably due to an experimental error in orientation. Ten of the sixteen cases with reduplications definitely follow the rule that the primary member of the pair is developed in accordance with the direction of the antero-posterior axis of the graft. Five others are doubtful with the probability that they conform. One is a peculiar case in which the primary limb is filiform and indeterminate, while the secondary has the asymmetric quality that, according to rule, the primary should have. This is apparently one of those exceptional cases of duplicate limbs in series[2]).

Table IV. Combined from Tables II and III, and showing the asymmetry or "laterality" of the limb, not with reference to its origin, but to the side of the host on which it is placed.

Origin and orientation of graft	Symmetry of Resulting Limbs							
	Single limbs harmonic or *"wirtsseiten-richtig"*	Single limbs disharmonic or *"wirtsseiten-verkehrt"*	Double limbs	Total positive	Indeterminate structures	Resorbed	Dead	Total
Hom. dd, aa, mm	3		4	7	3	2	7	19
Hom. dd, ap, ml		2		2		3	1	6
Hom. dv, ap, mm		11	1	12		14	5	31
Hom. dv, aa, ml	2			2		6	4	12
Het. dd, ap, mm		8	2	10	1	12	5	28
Het. dd, aa, ml	3		1	4		4	5	13
Het. dv, aa, mm	7	1 (?)*	8	16	8	16	20	60
Het. dv, ap, ml		1		1	1	9	5	16
Total	15	22 (23?)	16	54	13	66	52	185

* Due probably to an experimental error.

The results are so conclusive that it is difficult to see how any other interpretation can be put upon them. Starting with a given limb bud, say one from the right side, any one of eight different operations of the kind in question may be done with it. By this means any of the following results may be obtained, each in two different ways: a right limb on the right side, a right limb on the left side, a left limb on the right side or a left limb of the left side. The only thing varied in the different cases is the orien-

[1]) Cf. Harrison ('21), p. 4.
[2]) Cf. Swett ('24) and Harrison ('18) p. 441, and ('21) p. 77.

tation of the graft with respect to the body. It would seem that there could be no more simple way of showing the influence of surrounding parts on the development of the limb, and of showing that this influence acts in a certain direction. Yet PRZIBRAM denies such an influence.

Owing to the fact that the combinations of axial directions are different according as the medio-lateral axis is reversed or not (Table I), and owing also to the fact that the rules of symmetry for the transplantations with medio-lateral axis unchanged were originally phrased in terms of the orientation of the dorso-ventral axis and the origin of the bud, they do not hold in that form[1]) for the grafts in which the medio-lateral axis is reversed. It will be necessary, therefore, to restate them in terms of the orientation of the antero-posterior axis of the bud and the final asymmetry of the limb in relation to its seat of implantation (whether harmonic or disharmonic), thus putting them into a more general form which holds for all orientations. With this in mind the rules may be expressed as follows:

Rule 1. When the antero-posterior axis of the limb bud is reversed in grafting, the resulting limb has the asymmetry proper to the opposite side of the body from that on which it is placed, *i. e.*, it is disharmonic, whether originally taken from the same or the opposite side.

Rule 2. When the antero-posterior axis of the limb bud is not reversed in grafting, the resulting limb has the asymmetry proper to the side on which it is placed, i. e., it is harmonic, whether originally taken from the same or the opposite side.

Rule 3. When double limbs arise, the original member (the one first to begin its development) has its asymmetry fixed in accordance with rule 1 or 2, depending upon the orientation of the graft, while the secondary member is the mirror image of the first.

As implied in the statement of rules in the above form, it has been possible to go one step further than when they were first formulated. It is now certain that the material of which the limb bud is composed at the stages in question is polarized in one direction only, i. e., antero-posteriorly[2]), and that its other two axes are either indifferent or have their polarity so lightly fixed that it may be readily overcome by the influences from the surrounding region. Evidence for the equipotentiality of the system or, preferably, the totipotence of any sufficiently large fraction of it, remains unshaken and in fact has been strengthened by further experiments. The polarity shown

[1]) Cf. HARRISON ('21) p. 4.

[2]) PRZIBRAM ('24) assumes erroneously that both axes are determined from the beginning: "Die Querschnittsdetermination (GRÄPER) ist von Anfang an gegeben". Op. cit. p. 619.

by the system must therefore be represented by some quality of the minute structure of the blastema composing the limb bud, as already set forth previously[1]).

The next step in development is apparently the determination of the polarity of the dorso-ventral axis of the bud. We have as yet no final information on this point, but, according to a personal communication from Dr. Swett, this axis becomes fixed in *A. punctatum* certainly not later than stage 36[2]). In *Triton taeniatus*, however, as Brandt's work shows, the determination of the dorso-ventral axis of the fore-limb takes place before closure of the medullary folds, but whether the antero-posterior axis precedes it in this respect is uncertain. In the hind-limb bud of the anurans (*Rana* and *Bufo*) at the time it begins to elongate, the system is in a transitional state as far as the polarization of its axes is concerned, for Gräper finds that in many cases there is complete cross-sectional determination, while in others not. Here again it is uncertain whether the polarity of one axis becomes fixed before that of the other. In the blastema of the regenerating limb in *Triton taeniatus* according to Milojević the polarity of both dorso-ventral and antero-posterior axes is determined at the same time[3]). In all of the above cases, except in *Amblystoma punctatum*, it is also not known whether axial determination precedes or follows the fixed localization of germinal areas, i. e., whether we are dealing with equipotential systems with definite polarization.

In whatever way these questions may be settled in particular cases, the main point desired to be brougth out here is not that there is any magic connected with some particular axis, nor that the processes of axial determination take place always in the same order or under any constant influence. It is rather the fact that systems of the kind described exist at all in the developing organism. Such systems are already determined, *i. e.*, they are self differentiating as far as concerns the kind of organ that will develop out them; they are equipotential, in that there is no definitive localization of the constituent parts within them; their axial relations are in part but not wholly determined and the several axes may become polarized at different periods. The plate of ectoderm out of which the internal ear develops is also a system of this kind, as shown by an extensive series of experiments as yet unpublished. The fact, that the limb-

[1]) cf. Harrison ('21).

[2]) At stage 36 the external gills are beginning to sprout and the limb bud can be distinguished as a small nodule apart from the pronephros.

[3]) Milojević includes also the proximo-distal axis in his statment. His experiments (op. cit. p. 88) do not, however, seem to me to warrant this conclusion.

forming quality should become fixed before it is determined whether the limb is to be a rigth or left, or before it is determined which part of the rudiment will give rise to the radius, is surprising; but it is the recognition of conditions like this that will lead to a better understanding of the processes of development. Such facts show that the adult structures in question are represented in the germ not in the form of a rough mosaic, but rather in the intimate structure of the protoplasm of which the germ is made up. They show also that different qualities may be impressed upon the intimate structure at different times by various influences emanating from surrounding parts.

This conclusion accords well with views that have been previously expressed by many who have studied development by the experimental method — Driesch, Morgan, Conklin, Lillie, Braus, Spemann and others. It is even not unlike the views of Przibram ('21), who refers the symmetry and polarity relations of organisms and of systems within organisms to the arrangement of the particles as in a space lattice. Analogies of this kind will be found very useful in the study of embryology, though they inevitably lead us to the realization how vastly more complicated these relations are in organisms than in crystals.

V. Summary.

1. The present investigation is concerned with transplantations of the limb bud of *Amblystoma punctatum*, placed in a pocket under the skin of the flank of another embryo. After removal of its ectodermal covering, the graft is inserted into the pocket with its former inner surface turned outward or toward the skin and oriented in one of the four following ways: 1) disc from the same side of the body turned 180° about its dorso-ventral axis (Table I, hom, dd, ap. ml); 2) disc from the same side turned 180° about its antero-posterior axis (hom. dv, aa, ml); 3) disc from the opposite side of the body, not rotated (het. dd, aa, ml); 4) disc from the opposite site of the body rotated 180° about its transverse axis or center (het. dv. ap. ml).

2. Out of thirty-two cases that lived, appendages developed in ten (Table II). Seven of these were normal single limbs, one a defective single, one a rudimentary short process, and one a limb with a double hand.

3. The asymmetry or laterality of the single is in all eigth cases according to rule, depending on the orientation of the antero-posterior axis. When this is reversed, the resulting limb is disharmonic; when it is not reversed, the resulting limb is harmonic.

4. The primary member of the duplicity follows the latter rule; the duplicating member is the mirror image of the primary.

5. Owing to the different axial combinations found in the present transplantations as compared with the earlier ones, the rules of symmetry, as previously expressed, do not hold for the new series of experiments. The rules may, however, be expressed in more general form in terms of the antero-posterior axis of the bud and of the final asymmetry or laterality of the limb, so as then to hold for all orientations (p. 497).

6. The experiments show that the limb bud of *Amblystoma* in the stage in question has but one of its three axes polarized. Unlike the fore-limb bud of *Triton taeniatus* in corresponding stages (BRANDT), it has not yet passed to the stage of *Querschnittsdetermination.*

7. The eventual asymmetry of a limb is the resultant of two factors acting at rigth angles — one localized within the limb bud and acting in the direction of its antero-posterior axis, and one localized in the surrounding parts of the embryo and acting dorso-ventrally.

8. Previous experiments have shown that the fore-limb bud is an equipotential system (DRIESCH), and this conclusion has been supported by further experiments as yet unpublished. The axial relations of the limb bud must therefore be represented in some manner in the intimate structure of the protoplasm.

Zusammenfassung.

1. In der vorliegenden Untersuchung handelt es sich um Verpflanzungen der Vorderbeinknospe von *Amblystoma punctatum* in eine Hauttasche eines anderen Embryos. Nach Entfernung des Ectoderms wurde der Pfropf so gesetzt, daß seine früher innere Fläche nach außen, d. h. nach der Haut zu, gerichtet wurde, und zwar in einer der vier folgenden Orientierungen: 1. seitenrichtige Knospe, 180° um ihre dorsoventrale Achse gedreht (Tab. I, hom. dd, ap, ml); 2. seitenrichtige Knospe 180° um ihre antero-posteriore Achse gedreht (hom. dv, aa, ml); 3. seitenverkehrte Knospe, nicht gedreht (het. dd, aa, ml); 4. seitenverkehrte Knospe, 180° um die Querachse (d. h. Centrum der Scheibe) gedreht (het. dv, ap, ml).

2. Von 32 weiter lebenden Fällen entwickelten sich die Extremitäten in zehn (Tab. II). Sieben davon waren normale Vorderbeine, eine ein defektes einfaches Glied, eine ein kurzer rudimentärer Fortsatz und eine ein Glied mit doppelter Hand.

3. Die Seitenqualität der einfachen Beine erwies sich in allen Fällen, den Regeln gemäß, als abhängig von der Orientierung der antero-posterioren Achse der Knospe. Wenn diese umgedreht ist, wird das Bein wirtsseitenverkehrt; wenn nicht umgedreht, wirtsseitenrichtig.

4. Das primäre Glied der Doppelbildung unterliegt letzterer Regel; das sekundäre ist das Spiegelbild des ersten.

5. Dadurch, daß die Richtungen der Achsen der Pfröpfe in den vorliegenden Versuchen in anderen Kombinationen als in den früheren auftreten, gelten die Symmetrieregeln in der damaligen Ausdrucksweise für die neueren Versuche nicht. Die Regeln lassen sich, jedoch in einer verallgemeinerten Form, so ausdrücken, daß sie für beide Versuchsreihen, d. h. für sämtliche Orientierungen gelten (S. 497).

6. Die Versuche zeigen, daß die Polarität von nur einer Achse, der antero-posterioren, der Vorderbeinknospe von *Amblystoma punctatum* in den betreffenden Stadien fest determiniert ist. Darin erweist sie sich als verschieden von der Vorderbeinknospe von *Triton taeniatus*, wo nach BRANDT schon in den entsprechenden Stadien eine feste Querschnittsdetermination eingetreten ist.

7. Die eventuelle Seitenqualität der Extremität wird durch zwei senkrecht zueinander wirkende Faktoren bestimmt. Einer davon ist innerhalb der Beinknospe selbst lokalisiert und wirkt in eine antero-posteriore Richtung; der andere liegt in der näheren Umgebung des Pfropfes und wirkt dorso-ventral.

8. Aus früheren Versuchen wurde geschlossen, daß die Vorderbeinknospe ein äquipotentielles System ist (DRIESCH), und dieses wird durch weitere noch unveröffentlichte Versuche unterstützt. Die Achsenbeziehungen der Knospe müssen daher auf irgendeine Weise in der Intimstruktur des Protoplasmas vertreten sein.

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